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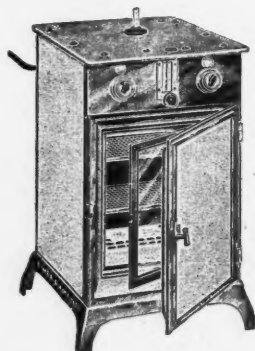
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SOIL SCIENCE

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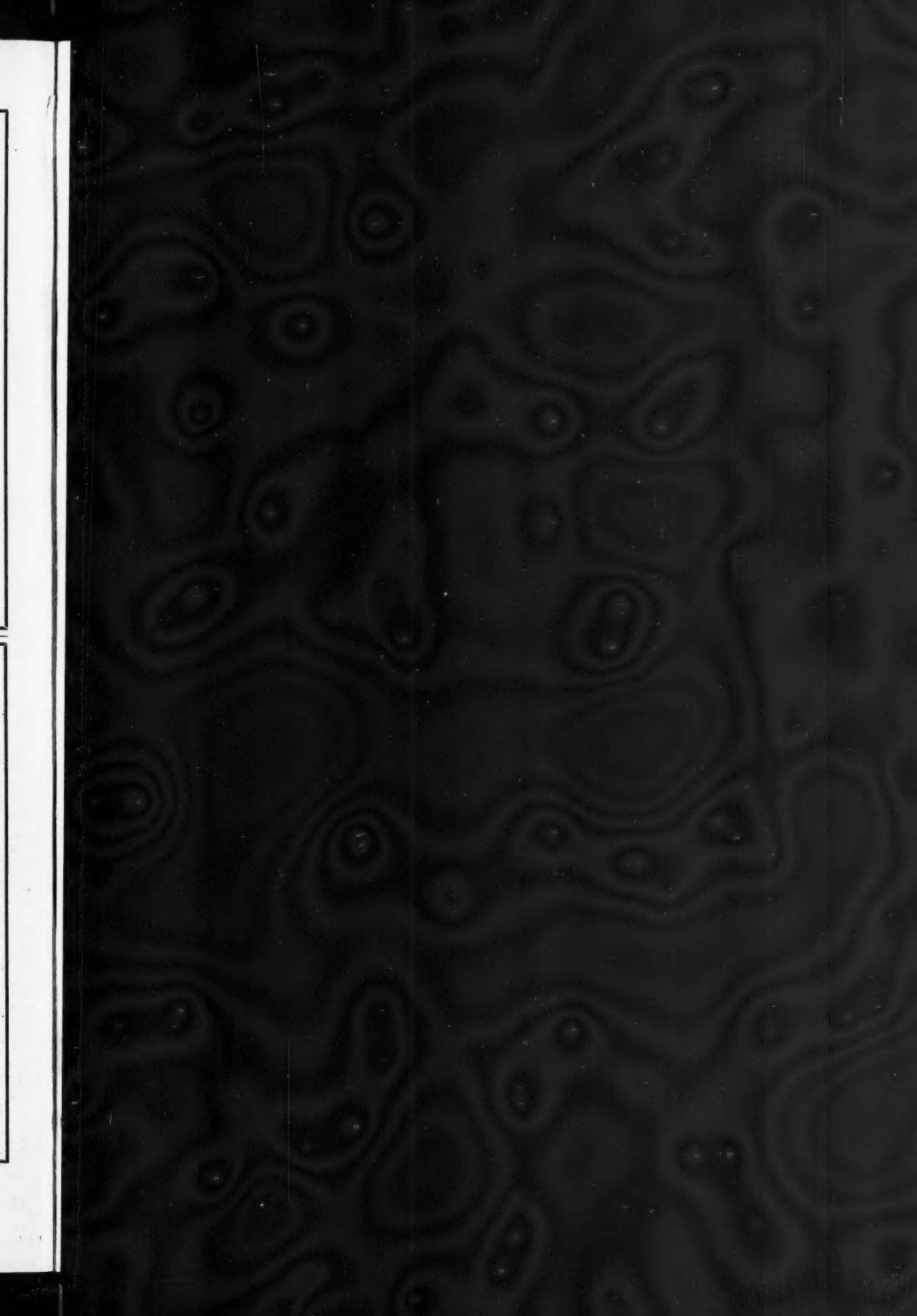
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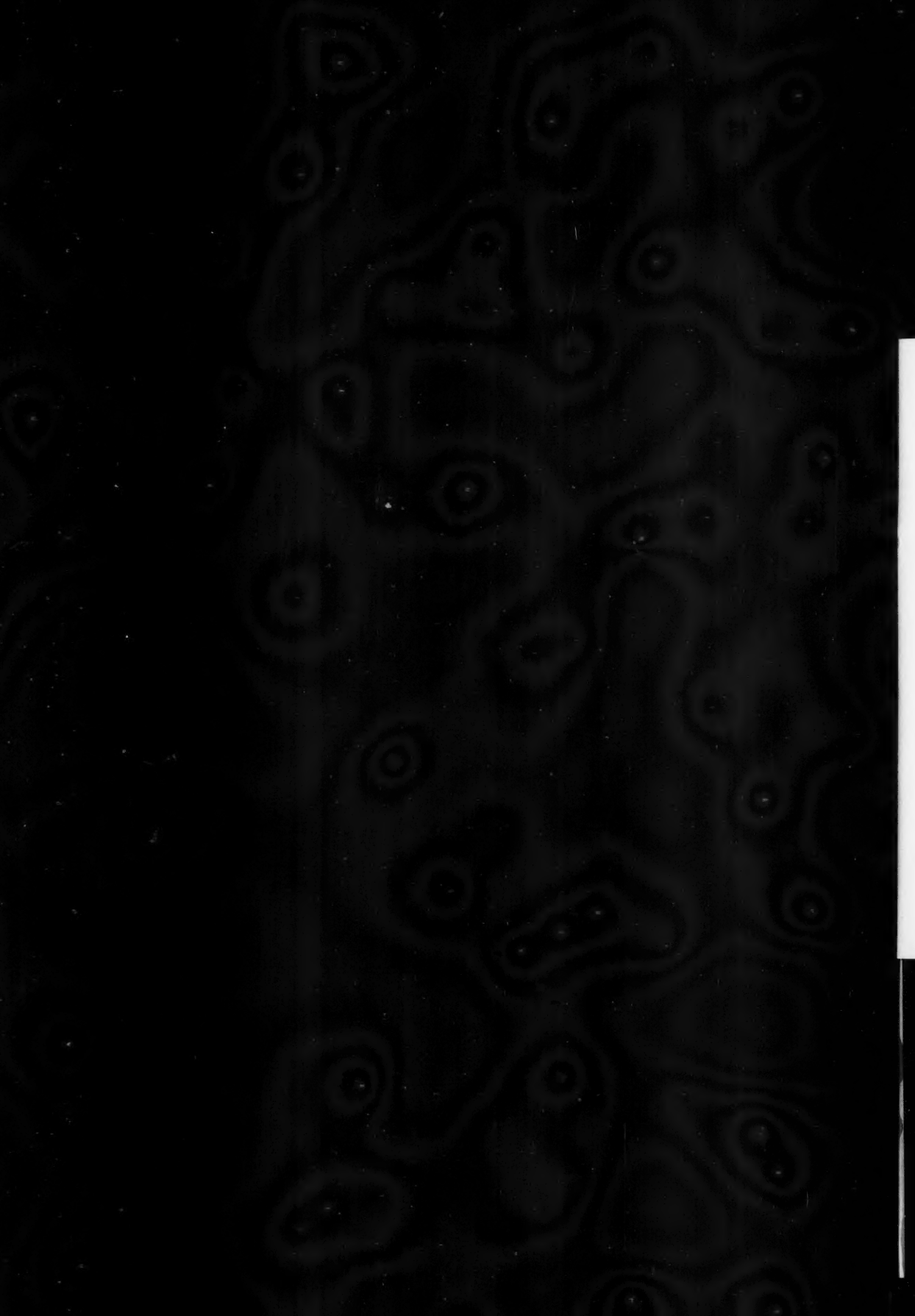
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ERRATA

p. 28, lines 16-18 should read:

Synthetic acid medium: 1000 cc. distilled water, 10 gm. dextrose, 5 gm. peptone, 1 gm. KH_2PO_4 , 0.5 gm. MgSO_4 , add acid to adjust pH to 4.0, 25 gm. agar.

p. 101, lines 11-12 should read:

(10) MELLOR, J. W. 1913 Higher Mathematics for Students of Chemistry and Physics. Longmans, New York.

p. 132, line 23, the following table should be inserted:

TABLE 4

Fluctuation of nitrogen in samples isolated in May, incubated in pots, and analyzed after monthly intervals

TIME OF ANALYSIS	INCREASE IN NITROGEN CONTENT			
	Light soil (initial N content was 36.1 mgm. per 100 gm.)		Heavy soil (initial N content was 37.5 mgm. per 100 gm.)	
	per cent	per cent	per cent	per cent
June.....	9.4	5.8	9.4	7.7
July.....	-4.1	-20.6	0.5	-8.3
August.....	-6.1	0.0	3.5	1.1
September.....	6.9	-1.1	8.3	4.8
October.....	10.0	10.2	5.1	6.9

p. 217, footnote should read:

¹ Paper No. 82 of the Journal Series, New Jersey Agricultural Experiment Stations, etc.

Chas. R.

THE EFFECT OF NODULE BACTERIA ON THE YIELD AND NITROGEN CONTENT OF CANNING PEAS¹

E. B. FRED AND O. C. BRYAN

University of Wisconsin

Received for publication January 11, 1922

INTRODUCTION

The study of the actual amount of nitrogen which inoculated legumes take from the air has almost invariably led to different results. These differences can be expected because of the variation in soil types and because of the nature of the different leguminous plants. A general discussion of the nitrogen sources of legumes is out of place here; the reader is referred to a report by Brown and Stallings (1). It may be pointed out, however, that according to the generally accepted statement, inoculated legumes increase the nitrogen supply of the soil. Accurate data supporting this statement and showing the amount of nitrogen which legumes add to the soil have been determined for certain soils and certain leguminous plants, but unfortunately little of this information has been obtained under field conditions. Because of the importance of combined nitrogen for soil fertility, it is desirable to measure carefully, under field conditions, the influence of legumes on the supply of nitrogenous compounds in the soil.

PROCEDURE AND RESULTS OF THIS STUDY

In a former paper (2) some results of field tests with Alaska peas, inoculated and uninoculated, on poor Plainfield sand and on rich Carrington silt loam soil were described. It was noted here that inoculation brought about an increase in the total yield of pea plants, in the yield of peas, and in the percentage of nitrogen in the peas.

In this paper, the influence of nodule bacteria on the yield of canning peas, and on the amount of nitrogen taken from the air will be considered. In the spring of 1921 two different types of Miami silt loam soils were selected for study. They were both slightly acid and had not been planted to peas for several years. One of the soils had been cultivated for only three years, contained 0.228 per cent of total nitrogen and was in a good state of fertility. The other soil had been cropped in grain and corn for the last five years and the total nitrogen in this soil was 0.180 per cent. Identical experiments, in so far as it was possible, were carried out on both of these soils. The plots were laid

¹ Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

out twelve feet wide on a uniform section of field and extended across the entire length of field. The peas were planted with a grain drill. The checks were planted first. All inoculations were made with pure cultures of bacteria. The same variety of peas, *Horsford*, was grown on both soils and was planted at the rate of 4 bushels per acre.

On May 25, about 30 days after the time of seeding, the plants were examined, but no difference in the size of the plants from the different plots was evident. At this time the roots of the inoculated plants on both soil types had numerous nodules while the uninoculated showed only a few. The effect of inoculation was already noticeable in the darker green color of these plants.

When the majority of the peas had reached the proper stage for canning, representative parts of the inoculated and uninoculated plots on the more fertile soil, $\frac{1}{8} \times \frac{1}{8}$ of an acre each, were measured off. The plants were carefully harvested, counted, and weighed. These data as well as the nitrogen contents are given in table 1. Comparison of weights shows that in spite of the decrease² in the number of plants, there were more pounds of tops per acre on the inoculated than on the uninoculated plot. It is true that no gain in weight of pods and peas could be noted and yet, on analysis, a substantial increase in the percentage of nitrogen was seen. If calculated as pounds per acre, the increase in yield above that of the control amounts to 217.4 pound of dry tissue and a gain of 37.25 pound of nitrogen. Since some of the plants in the uninoculated plot became infected this difference in nitrogen between the inoculated and uninoculated is below the actual amount of nitrogen fixed from the air.

When the peas were ready to can representative parts of the inoculated and uninoculated plots on the less fertile soil, $\frac{1}{8} \times \frac{1}{8}$ of an acre each, were measured off and the entire crop was harvested. The plants were dug up carefully; the roots and nodules were freed from the soil. Because of its open texture, the soil could be easily removed from the roots without a great loss of nodules. The nodules were picked from the roots and analyzed. The results of the analysis of the tops and roots, peas and hulls, and the nodules are given in table 2.

Although not great, there was a well defined difference between the inoculated and uninoculated plants. The peas treated with nodule bacteria were somewhat taller and of a darker green color. The total yield of peas was only about one-half that obtained from the more fertile soil. At best, the yield of peas on this soil was not more than half of a normal crop.

Here, as in the previous test, there are fewer plants on the inoculated section than on the uninoculated. The effect of bacteria on the yield and on the nitrogen content of the plants is readily seen from the figures in this table. With the exception of the seed, there is a well defined gain in yield of the inoculated plants as compared with the uninoculated. It is in the percentage

² The decrease in number of plants in the inoculated plots is no doubt due to the number of seed planted. Treatment of the pea seed with a water suspension of the nodule bacteria caused them to swell. Similar results were obtained in a second test.

of nitrogen in the different parts of the plant that the result of inoculation becomes most noticeable; there is a gain in the tops and roots, in the hulls, and also in the peas. Although almost without effect on the yield of crop, the nodule bacteria brought about a substantial increase in the percentage of nitrogen.

For the each acre treated, the treated plants show a net gain of 179.0 pounds of tops and roots and 51.5 pounds of hulls. The gain in nitrogen of the inoculated crop over that of the uninoculated crop was 13.2 pounds.

TABLE 1

The yield and amount of nitrogen in canning peas grown with and without inoculation on Miami silt loam soil

TREATMENT	NUMBER OF PLANTS	TOTAL YIELD (DRY)	DRY WEIGHT		NITROGEN	
			Tops	Peas and pods	Tops	Peas and pods
		gm.	gm.	gm.	per cent	per cent
Inoculated	575	2055	1715	340	3.67	4.42
Uninoculated	711	1858	1518	340	2.17	3.31
Gain due to inoculation	-136	+197	+197	0	+1.50	+1.11

TABLE 2

The yield and amount of nitrogen in inoculated and uninoculated canning peas on poor Miami silt loam soil

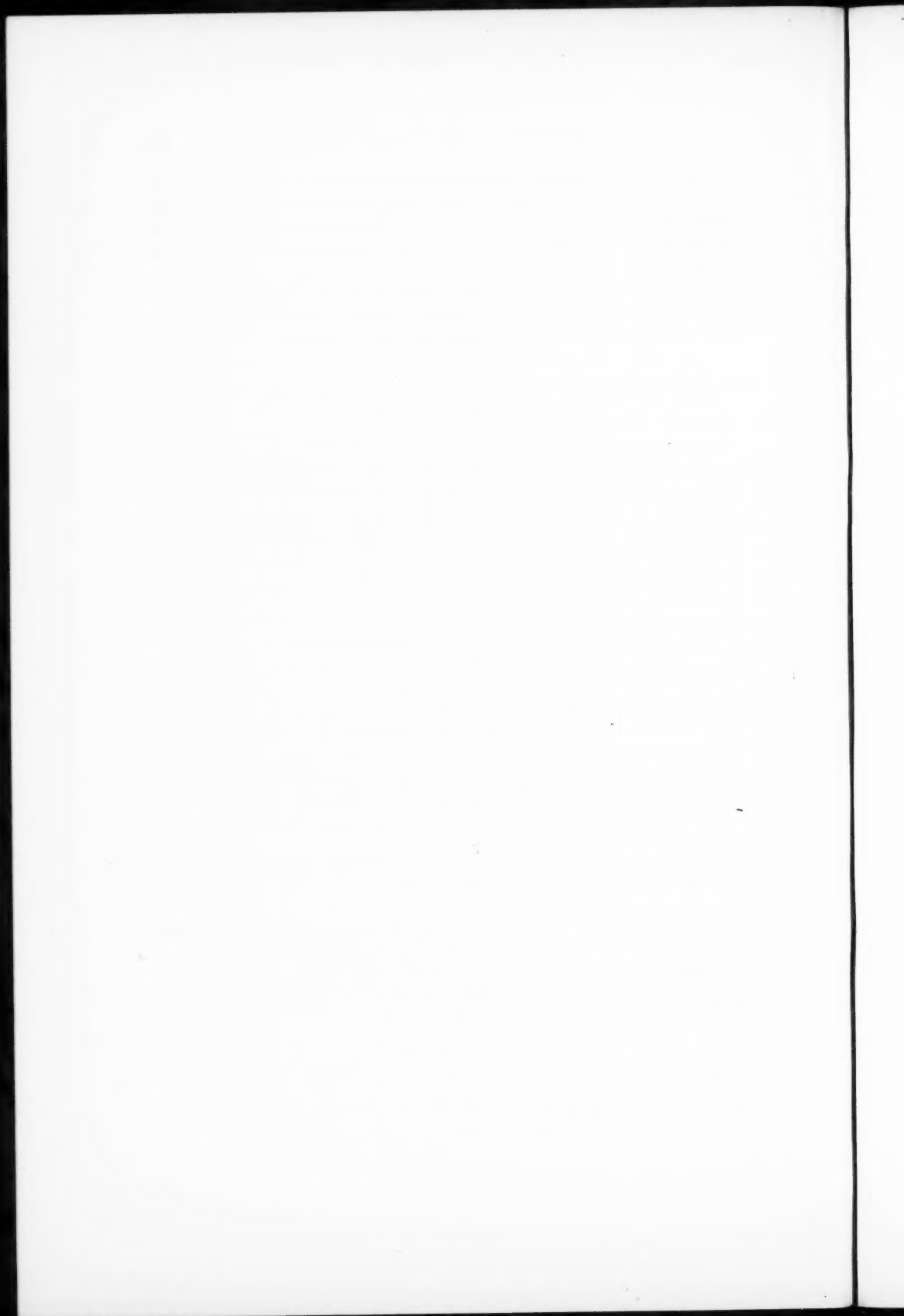
TREATMENT	NUMBER OF PLANTS	YIELD (DRY WEIGHT)					NITROGEN			
		Total	Tops and roots	Hulls	Peas	Nodules	Tops and roots	Hulls	Peas	Nodules
		gm.	gm.	gm.	gm.	gm.	per cent	per cent	per cent	per cent
Inoculated	545	1480	1162	210	94	14.0	1.97	2.34	4.46	1.49
Uninoculated	572	1256	1000	163	93	0.0	1.44	1.61	3.50	0.0*
Gain due to inoculation	-27	+224	+162	+47	+1	+14.0	+0.53	+0.73	+0.96	+1.49

* A few nodules present but not enough for analysis.

This beneficial effect of inoculation on garden peas, which is noticeable not only in the total nitrogen but also in the yield, is probably much greater than the figures indicate. As is the case with nearly all field tests, the uninoculated plants were not entirely without nodules and accordingly were benefiting from the bacteria naturally present in the soil.

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- (1) BROWN, P. E., AND STALLINGS, J. H. 1921 Inoculated legumes as nitrogenous fertilizers. *In Soil Sci.*, vol. 12, p. 365-407.
- (2) FRED, E. B., WRIGHT, W. H., AND FRAZIER, W. C. 1921 Field tests on the inoculation of canning peas. *In Soil Sci.*, vol. 11, p. 479-485.



THE FORMATION OF NODULES BY DIFFERENT VARIETIES OF SOYBEANS¹

E. B. FRED AND O. C. BRYAN

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Received for publication July 10, 1922

Variation in nodule formation among different varieties of soybeans has been reported from different parts of Wisconsin. In many cases a certain variety has failed to show inoculation when treated with a culture of known origin, while among different varieties growing side by side one has been inoculated and the other has remained free or practically free from nodules. Similar observations have been made in other parts of the United States.

Vorhees (2) of New Jersey found that in a mixture of Brown and Haberlandt grown on medium heavy clay loam soil the two varieties did not show the same nodule formation. Although their root systems were closely associated in the soil, only the Brown variety produced nodules. Morse (2), in discussing Voorhees' paper, reports that at the West Tennessee Experiment Station the Acme and Tokio varieties of soybeans failed to form nodules, while the Mammoth variety under the same conditions formed many nodules. Similar results were obtained the next year.

Leonard (1), on the other hand, as a result of laboratory and green-house tests concluded that a culture of bacteria isolated from a single strain of soybeans will form nodules on other varieties. In his experiments the organism was isolated from the Medium Yellow variety of soybean. Nineteen different varieties of soybeans were studied, including Amherst, Arlington, Barchet, Chernie, Chestnut, Cloud, Guelph, Haberlandt, Hope, Ito San, Jet Manhattan, Medium Yellow, Pekin, Taha, Virginia and Wilson.

It seems from these reports that the ability to form nodules varies among the different kinds of soybeans. Apparently certain varieties are more easily inoculated than others. It is also true that the time of ripening of one variety may influence the infection and subsequent formation of nodules. For instance, an early and late variety may show differences in inoculation as a result of variation in plant food at different times of the year. Repeated investigations have proved that the formation of nodules is influenced to a considerable degree by the reaction and salt concentration of the soil water as well as by other factors.

Because of the importance of soybeans in Wisconsin, it seemed of interest to measure under field and laboratory conditions the effect of different strains of soybean bacteria on the assimilation of nitrogen by different varieties of

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soybean plants and to contrast pure-culture inoculation with soil inoculation. The results of these studies are presented in this paper.

Pure cultures of the different strains of bacteria were obtained from known varieties of soybeans, and their ability to cause inoculation was determined previous to these experiments. The soil used for inoculation was from a field on the Experiment Station Farm where well inoculated soybeans had been grown for several years. Although the records are not available, it is highly probable that the varieties grown on this soil were Wisconsin Black and Ito San soybeans.

GREENHOUSE TESTS

Glazed earthenware pots were filled with pure white silica sand to which had been added an excess of CaCO_3 . These were sterilized and then planted in duplicate for each culture to Manchu, Mammoth Yellow, Wisconsin Black,

TABLE 1
Effect of different strains of soybean bacteria on the nitrogen content of different varieties of soybeans, greenhouse test

SOURCE OF BACTERIA	NITROGEN CONTENT OF TOPS, ROOTS AND NODULES			
	Manchu	Mammoth Yellow	Wisconsin Black	Medium Early Green
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Not inoculated.....	1.85	1.64	1.35	1.42
Mammoth yellow.....	2.85	3.13	2.95	
Medium yellow.....	3.02	2.95	2.74	
Manchu.....	3.02	3.02	2.68	
Haberlandt.....	3.01	2.85	2.76	2.93
Soil from soybean field.....	2.37	2.79	2.45	2.87

and Medium Early Green soybeans, which had been treated immediately preceding planting with either the desired pure culture or with soil. For example, Manchu soybeans were inoculated with bacteria from Mammoth Yellow, Medium Yellow, Haberlandt, and Manchu varieties of soybean. Nitrogen-free plant food was added at intervals in quantities sufficient for plant growth.

Within three weeks after planting, a well defined contrast was noticeable in size and color of the inoculated and uninoculated plants. The uninoculated plants were smaller and at the end of five to six weeks decidedly yellow. At this stage the plants were washed from the sand and their appearance as to size, color, and inoculation was carefully noted.

The combined tissue of the two parallel pots was used for analysis. Table 1 gives the percentage of nitrogen in the dry soybean plants, tops, roots, and nodules. Under the conditions of this experiment all indications are that pure cultures of bacteria from the nodules of Mammoth Yellow, Manchu, Haberlandt, or Medium Yellow soybeans may be used interchangeably on

the different varieties of the soybeans and are almost equally effective in assimilating nitrogen.

The same conclusions are to be drawn from the use of well inoculated soil as a source for soybean bacteria. The general appearance of the soil inoculated plants as to size and color of plants and number of nodules was equally as good as that of the plants inoculated with pure cultures, and on analysis the tissue showed a comparable percentage of nitrogen fixed.

FIELD TESTS

A plot of sandy soil on the farm of Mr. J. R. Williams at Montello was selected for this study. This soil had never grown soybeans. It had been cropped in corn and grain for the last 6 or 8 years and was in rather poor

TABLE 2

Effect of different strains of soybean bacteria on the dry weight and percentage of nitrogen of different varieties in soybean field test

SOURCE OF BACTERIA	WEIGHT AND NITROGEN CONTENT OF DRY STALKS AND PODS							
	Manchu		Wisconsin Black		Ito San		Black Eye-brow	
	lbs.	per cent	lbs.	per cent	lbs.	per cent	lbs.	per cent
Not inoculated.....	306.5	5.45	613.0	6.04	540.2		824.7	5.44
Manchu.....	573.3	5.36	949.0	6.10	637.2	6.0	1217.2	6.23
Mammoth Yellow.....	641.7	5.45	826.9	6.45	692.4		1226.0	6.45
Haberlandt.....	793.8	5.40	959.2	6.48	597.6	5.96	1226.0	6.61

state of fertility. No fertilizer or manure was added. The reaction was medium acid. On May 25 the plots were laid out; each plot consisted of two 30-inch rows twelve rods long, planted to soybeans with a small hand drill. All check plots were planted first; Ito San, Manchu, Mammoth Yellow, Wisconsin Black, Black Eyebrow, and Hollybrook varieties of soybeans were used. Then quantities of each variety of seed sufficient to plant a single plot were inoculated with the Manchu strain of soybean bacteria. When these had been planted the hopper of the planter was washed with water and the operation repeated for the seed which was inoculated with the Mammoth Yellow and the Haberlandt strain of bacteria respectively. On September 27, one-thousandth of an acre was harvested from each plot, bagged, and expressed to Madison. There were practically no nodules on any plants in the check plots, while all plants in the treated plots showed numerous nodules. The plants in the treated plots were in all cases larger and greener than those of the checks.

The oven-dry weights of the pods and stalks together with the percentage of nitrogen are recorded in table 2. The Mammoth Yellow and Haberlandt varieties were too late in ripening to be of any value and were not analyzed.

The general appearances were about the same for all varieties of soybeans

regardless of the source of the culture of bacteria used to inoculate. Apparently one strain of bacteria was as efficient as another. The uninoculated soybeans produced a much smaller amount of dry seed and also a lower percentage of nitrogen.

From the results of these laboratory and field studies, there is no evidence to conclude that the nodule bacteria of soybeans are highly specific, but on the contrary it was found that the bacteria of one variety will readily infect another. Variation in nodule formation as seen under field conditions must be due to some factor other than difference in bacteria.

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- (1) LEONARD, L. T. 1916. Variation in nodule formation. *In* Jour. Amer. Soc. Agron., v., 8, p. 116-118.
- (2) VOORHEES, J. H. 1915. Variations in soybean inoculation. *In* Jour. Amer. Soc. Agron., v. 7, p. 139-140.

SULFUR AS AN IMPORTANT FERTILITY ELEMENT¹

C. O. SWANSON AND W. L. LATSHAW²

Kansas Agricultural Experiment Station

Received for publication July 1, 1922

Sulfur is one of the essential elements of plant growth. An element is essential either because it furnishes building material or performs some important physiological function, or both. Sulfur is one of the elements of protein compounds and because of the intimate connection of these compounds with life processes it probably performs physiological functions in connection with the formation of compounds that do not contain sulfur. It follows therefore that profitable crop production is not possible in the absence of an adequate supply of available sulfur any more than it is possible in the absence of an adequate supply of available nitrogen, phosphorus or potassium.

The possibility of sulfur being a limiting element in crop production has only lately been recognized. References to the literature may be found in papers by Lipman and McLean (4), McLean (5), and Ames and Boltz (1). Delay of this recognition was due mainly to incorrect methods of analyses of sulfur both in crops and in the soil. Hilgard (3) gives the following averages for percentages of sulfur in soil: Humid region of the United States, 0.02; transition region, 0.008; arid region, 0.02. These figures of Hilgard are based on the old method for sulfur determination. Shedd (7) found an average of 0.02 per cent sulfur in eight Kentucky soils. Ames and Boltz (1) found that soils in Ohio averaged from 330 to 1112 pounds sulfur per acre surface soil six inches deep. Reimer and Tartar (6) found 0.038, 0.032, 0.02, 0.038, 0.021, 0.027, 0.037, 0.028, 0.029, 0.015, 0.024 and 0.036 per cent of sulfur in Oregon soils. These percentages from the several states are not so widely different from the results obtained on Kansas soils.

The differences in results obtained on soils by the older methods are not as great as those obtained on plant substances. This is shown by the following comparison of analyses by Hart and Peterson (2) with the earlier analyses made by Wolff (10).

The greatest error caused by the old methods of analysis was due to the fact that most of the sulfur was thought to be present in the leaves and stems of the plant which are returned largely to the soil in one form or another, and a comparatively small amount in the seeds, the portion of the plant usually sold from the farm. The amount of sulfur removed from the soil by selling grain according to the old methods of analysis was so small that the loss was more than made up by the addition of sulfur in rain. The recent methods of

¹ Contribution No. 99 from the Department of Chemistry of the Kansas State Agricultural Experiment Station.

² A preliminary report of this investigation was read at the spring meeting of American Chemical Society, St. Louis, 1920. Released for publication in *SOIL SCIENCE* by courtesy of H. E. Howe, Editor of the *Journal of Industrial and Engineering Chemistry*.

analysis show that the total sulfur removed in grain is more than that present in the stalks of such crops as corn and wheat.

Another reason why the importance of sulfur as a possible limiting element of crop production was not earlier recognized is that sulfur is added to the soil in several of the carriers of the element present in commercial fertilizers. Ammonium sulfate, potassium sulfate and acid phosphate are well known examples of such carriers of sulfur. The calcium sulfate present in a ton of ordinary 16-per cent acid phosphate contains about 200 pounds of sulfur. One hundred pounds of such acid phosphate would then contain approximately as much sulfur as is present in the grain and straw of a 50-bushel wheat crop. Acid phosphate is one of the most important ingredients in commercial fertilizers. Ammonium sulfate and potassium sulfate are also important. Gypsum as land plaster has long been used as a soil amendment. It is thus easy to see that sulfur has been incidentally applied to worn soils in connection with the use of nitrogen, phosphorus and potassium.

TABLE 1
Sulfur content of common farm crops

CROP	SULFUR IN PLANT (HART AND PETERSON)	SULFUR IN PLANT (WOLFE)
	<i>per cent</i>	<i>per cent</i>
Alfalfa hay	0.287	0.170
Red clover	0.164	0.089
Maize, grain	0.170	0.004
Maize, stover	0.126	0.113
Oats, grain	0.189	0.002
Oats, straw	0.195	0.092
Timothy	0.190	0.078
Wheat, grain	0.170	0.003
Wheat, straw	0.119	0.053

In a previous investigation at the Kansas State Agricultural College (8) a study was made of the sulfur content of virgin soils in comparison with soils that had been cropped to grain. All the soils, with one exception, included in that study were from the eastern or humid part of the state and almost all were upland soils.

With the exception of two pairs, the soils then studied were not from contiguous fields. The average sulfur content of the cropped fields, compared with the average sulfur content of the fields in virgin sod showed that the cropped soils had 40 per cent less sulfur than the soils in virgin sod. By the same comparison it was shown that the loss of carbon and nitrogen had been 38 per cent for each. The average difference in phosphorus content of the virgin soil compared with the cropped soil was within the experimental error of phosphorus determination consequently it could not be stated that there had been a loss of phosphorus.

These facts warranted further investigation. In conducting the investigation on the effect of alfalfa on the fertility elements of the soil in comparison with grain crops (9), soil samples were collected from 96 fields located in different parts of Kansas. These samples furnished excellent material for the further study of the sulfur problem. The plan followed in collecting these

samples was to locate, in different parts of the state, alfalfa fields which had been in this crop for a long time, most of them from 20 to 30 years. Near these alfalfa fields were usually found soils of the same type which had been cropped to grain continuously since broken. As a rule this time was about 40 years. Near these fields cropped to grain or alfalfa, were nearly always found soils in virgin sod. In the central and western part of the state nearly all the fields (alfalfa, grain and sod) were on bottom land. The soil samples were taken in four strata: 0-7 inches, 7-20 inches, 20-40 inches, and 40-80 inches. For the work on sulfur only the surface soils were used.

Sulfur was determined in these soil samples by two methods: The Osborne method using sodium peroxide to oxidize the sulfur; and by a method devised in this laboratory, substituting magnesium nitrate for sodium peroxide. The sodium peroxide method gives good results in the hands of an experienced analyst, but it has a number of disadvantages well known to all who have used the method. The use of magnesium nitrate instead of sodium peroxide has several distinct advantages. The method used as is follows:

The magnesium nitrate solution is made by dissolving 320 gm. of calcined magnesia in nitric acid. The magnesia must be in excess. Boil, filter and dilute to 2000 cc. Add 10 cc. of this magnesium nitrate solution to 5-gm. portions of soil contained in 75-cc. porcelain dishes. Mix soil and magnesium nitrate solution well; evaporate on electric hot plate. Ignite to red heat in electric muffle furnace; add enough water to moisten; loosen and pulverize with pestle without removing from porcelain dish; add 10 cc. of concentrated hydrochloric acid and evaporate to dryness on hot plate. Moisten with water; add 10 cc. of concentrated hydrochloric acid; heat to boiling; and filter and wash on a Hirsch-Büchner funnel, using medium suction, into 250 cc. beakers. Heat to the boiling point on the electric hot plate; add, drop by drop, 5 cc. of a 10-per cent solution of BaCl_2 ; digest 1-2 hours; allow to stand over night or an equivalent length of time; filter on tarred Gooch crucible; ignite and weigh as usual. It is obvious that blank determinations must be made on all reagents used.

One advantage of the magnesium nitrate method is that the phosphorus if wanted, can be determined on the filtrate from the sulfur determination. The method is to neutralize with ammonium hydroxide; acidify with nitric acid; evaporate to about 25 cc.; add 5 to 10 gm. ammonium nitrate; raise the temperature to the desired degree and precipitate the phosphorus with the molybdate solution.

The average sulfur content indicated by 96 determinations made by each of these methods was 0.035 per cent for the magnesium nitrate method and 0.0345 per cent for the sodium peroxide method. This shows that the magnesium nitrate method was as reliable in the aggregate as the sodium peroxide method. It was noticed that the determinations with the magnesium nitrate method gave better duplicates in the hands of different analysts and this made it appear that individual results were therefore more reliable. For this reason the results obtained by the magnesium nitrate method are used in the tables of this report.

The results of the analyses of the soils are found in table 2. Since these samples were taken from all parts of the state, differing greatly in climatic

TABLE 2
Analyses of soils

SAMPLE NO.	COUNTY	CROP	SULFUR	NITROGEN	CARBON	PHOS-PHORUS
Humid section—Alfalfa						
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1768	Brown	Alfalfa 28 years	0.044	0.211	2.28	0.063
1897	Butler	Alfalfa 12 years	0.026	0.161	1.71	0.038
1900	Chase	Alfalfa 25 years	0.032	0.201	2.23	0.039
1874	Dickinson	Alfalfa 20 years	0.028	0.168	1.82	0.048
1877	Dickinson	Alfalfa 20 years	0.026	0.177	2.00	0.044
1880	Dickinson	Alfalfa 25 years	0.032	0.201	2.51	0.091
1882	Dickinson	Alfalfa 15 years	0.021	0.157	2.27	0.052
1890	Harvey	Alfalfa 11 years	0.023	0.161	1.91	0.041
1893	Harvey	Alfalfa 14 years	0.028	0.200	2.31	0.043
1316	Leavenworth	Alfalfa 8 years	0.033	0.163	1.61	0.043
1365	Nemaha	Alfalfa 20 years	0.030	0.170	1.69	0.039
Average.....			0.029	0.179	2.03	0.049
Humid section—Virgin sod						
1770	Brown	Pasture, blue grass	0.041	0.228	2.86	0.061
1898	Butler	Pasture, native	0.030	0.196	2.35	0.035
1903	Chase	Meadow, blue stem	0.034	0.201	2.48	0.058
1876	Dickinson	Meadow, blue stem	0.026	0.204	2.46	0.048
1879	Dickinson	Native, blue stem	0.038	0.204	2.63	0.061
1881	Dickinson	Native, blue stem	0.035	0.209	3.03	0.087
1891	Harvey	Native, blue stem	0.031	0.189	2.28	0.056
1894	Harvey	Native, pasture	0.031	0.222	2.66	0.045
1319	Leavenworth	Native, blue stem	0.047	0.296	3.76	0.061
1367	Nemaha	Native, pasture	0.036	0.181	1.97	0.038
Average.....			0.035	0.193	2.65	0.055
Humid section—Cropped to grain						
1769	Brown	Grain, 50 years	0.027	0.160	1.94	0.048
1896	Butler	Corn and wheat, 32 years	0.024	0.139	1.54	0.027
1901	Chase	Corn and wheat, 40 years	0.027	0.133	1.80	0.042
1875	Dickinson	Small grain, 33 years	0.030	0.140	1.82	0.057
1878	Dickinson	Wheat and oats, 40 years	0.023	0.163	2.00	0.048
1883	Dickinson	Wheat and kaffir, 35 years	0.028	0.155	2.06	0.045
1892	Harvey	Wheat and corn, 25 years	0.023	0.140	1.12	0.053
1895	Harvey	Wheat and corn, 43 years	0.022	0.124	1.23	0.036
1317	Leavenworth	Grain, corn 40 years	0.035	0.174	1.87	0.048
1766	Nemaha	Grain, 48 years	0.024	0.102	1.13	0.040
Average.....			0.026	0.143	1.65	0.044

TABLE 2—Continued

SAMPLE NO.	COUNTY	CROP	SULFUR	NITROGEN	CARBON	PHOS-PHORUS
Subhumid section—Alfalfa						
1884	Barton	Alfalfa, 15 years	0.031	0.159	1.91	0.054
1771	Mitchell	Alfalfa, 21 years	0.044	0.204	2.42	0.075
1774	Mitchell	Alfalfa, 21 years	0.076	0.269	3.23	0.069
1779	Mitchell	Alfalfa, 23 years	0.037	0.160	1.26	0.079
1783	Osborne	Alfalfa, 20 years	0.041	0.184	2.30	0.060
1787	Osborne	Alfalfa, 33 years	0.049	0.196	2.22	0.070
1887	Pawnee	Alfalfa, 28 years	0.035	0.221	2.56	0.053
Average			0.045	0.199	2.27	0.066
Sub-humid section—Virgin sod						
1885	Barton	Native grass	0.021	0.106	1.28	0.052
1773	Mitchell	Native pasture	0.042	0.238	3.14	0.063
1776	Mitchell	Native, grass	0.085	0.290	2.89	0.105
1778	Mitchell	Native grass	0.034	0.180	1.87	0.068
1780	Mitchell	Native, pasture	0.040	0.153	1.78	0.055
1784	Osborne	Native, pasture	0.051	0.250	3.22	0.068
1786	Osborne	Native, grass	0.053	0.232	3.71	0.106
1789	Osborne	Native, timber	0.039	0.220	3.11	0.100
1888	Pawnee	Buffalo grass	0.033	0.214	2.53	0.059
Average			0.044	0.209	2.61	0.075
Sub-humid section—Cropped to grain						
1886	Barton	Wheat and corn, 30 years	0.022	0.137	1.68	0.047
1772	Mitchell	Wheat and corn, 30 years	0.040	0.180	2.18	0.072
1775	Mitchell	Wheat and corn, 23 years	0.091	0.186	2.09	0.096
1777	Mitchell	Wheat and corn, 30 years	0.033	0.129	1.42	0.065
1781	Mitchell	Wheat and corn, 40 years	0.026	0.115	1.34	0.057
1782	Osborne	Wheat and corn, 40 years	0.037	0.134	1.66	0.067
1785	Osborne	Wheat and corn, 35 years	0.048	0.211	2.65	0.103
1788	Osborne	Corn, 40 years	0.049	0.143	1.77	0.080
1789	Pawnee	Corn and kafir, 20 years	0.036	0.201	2.11	0.093
Average			0.042	0.160	1.88	0.076
Semi-arid section—Alfalfa						
1299	Finney	Alfalfa, 20 years	0.034	0.168	1.61	0.076
1303	Finney	Alfalfa, 27 years	0.059	0.200	1.93	0.085
1304	Finney	Alfalfa, 27 years	0.034	0.178	1.72	0.064
1306	Finney	Alfalfa, 30 years	0.055	0.192	2.04	0.078
1310	Ford	Alfalfa, 30 years	0.036	0.210	2.08	0.082

TABLE 2—*Concluded*

SAMPLE NO.	COUNTY	CROP	SULFUR	NITROGEN	CARBON	PHOS-PHORUS
Semi-arid section—Alfalfa—Continued						
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1313	Ford	Alfalfa, 10 years	0.027	0.156	3.66	0.082
1794	Trego	Alfalfa, 20 years	0.025	0.131	1.06	0.072
1798	Gove	Alfalfa, 15 years	0.036	0.210	2.23	0.100
1800	Gove	Alfalfa, 30 years	0.034	0.184	1.90	0.093
1802	Gove	Alfalfa, 20 years	0.030	0.133	1.44	0.098
1806	Sheridan	Alfalfa, 20 years	0.033	0.187	1.83	0.093
1809	Sheridan	Alfalfa, 20 years	0.027	0.153	1.63	0.084
1811	Wallace	Alfalfa, 25 years	0.046	0.182	1.76	0.099
Average			0.037	0.176	1.91	0.085
Semi-arid section—Virgin sod						
1300	Finney	Buffalo grass	0.027	0.137	1.94	0.077
1302	Finney	Buffalo grass	0.031	0.135	1.68	0.082
1308	Finney	Buffalo grass	0.015	0.099	0.89	0.068
1315	Ford	Buffalo grass	0.032	0.152	1.74	
1797	Gove	Buffalo grass	0.027	0.154	1.84	0.084
1799	Gove	Buffalo grass	0.032	0.188	2.16	0.086
1803	Gove	Buffalo grass	0.029	0.116	1.27	0.080
1807	Sheridan	Buffalo grass	0.034	0.182	2.27	0.064
1810	Sheridan	Buffalo grass	0.028	0.157	1.82	0.098
1812	Wallace	Buffalo grass	0.033	0.151	1.89	0.080
Average			0.028	0.149	1.78	0.080
Semi-arid section—Cropped to grain						
1301	Finney	Wheat and Sudan grass, 20 years	0.042	0.134	1.76	0.070
1305	Finney	Wheat and grain, 27 years	0.019	0.079	1.04	0.053
1307	Finney	Wheat and grain, 27 years	0.016	0.097	0.98	0.062
1312	Ford	Wheat and grain, 30 years	0.031	0.136	1.82	0.075
1314	Ford	Wheat and grain, 30 years	0.021	0.118	1.48	0.063
1795	Trego	Wheat, unknown	0.019	0.160	1.84	0.073
1796	Gove	Wheat, 30 years	0.019	0.128	1.35	0.105
1801	Gove	Wheat and grain, 15 years	0.025	0.121	1.21	0.090
1804	Gove	Wheat, 30 years	0.033	0.118	1.31	0.099
1808	Sheridan	Wheat and grain, 20 years	0.024	0.118	1.14	0.082
Average			0.025	0.121	1.39	0.077

conditions, it was thought best to group the results under three heads. Under the humid section are classed all soils taken where the average rainfall is 30 inches or more; under the sub-humid section, those taken where the average rainfall is between 30 and 22 inches; and under the semi-arid section, those

taken where the average rainfall is less than 22 inches. The percentages of nitrogen, carbon and phosphorus are taken from the study last mentioned (9).

The results on sulfur showed great variation in different groups but not more so than the results on nitrogen or phosphorus. It is only when the average for each group within the different sections are considered that any conclusions can be drawn. These averages were used to calculate the pounds per acre assuming two million pounds for the surface seven inches. The results obtained by this calculation are found in table 3.

In examining the results of the sulfur determinations it was noticed that three soils from the semi-arid section had an abnormally high content of sulfur. Soil 1309 was 0.149 per cent sulfur. This sample was taken on bottom soil near the Arkansas river which had been flooded occasionally. Soil 1311 was 0.065 per cent sulfur. This sample was taken on virgin sod near the railroad.

TABLE 3
Average plant food elements per acre in the surface soil

SECTION	CROP	SULFUR	NITROGEN	CARBON	PHOSPHORUS
		<i>pounds</i>	<i>pounds</i>	<i>pounds</i>	<i>pounds</i>
Humid	Alfalfa	580	3,580	40,600	980
	Virgin sod	700	3,860	53,000	1,100
	Grain	520	2,860	33,000	880
Sub-humid	Alfalfa	900	3,980	45,400	1,320
	Virgin sod	880	4,180	52,200	1,500
	Grain	840	3,200	37,600	1,520
Semi-arid	Alfalfa	740	3,520	38,200	1,700
	Virgin sod	560	2,980	35,600	1,600
	Grain	500	2,420	27,800	1,540

Soil 1805 was 0.111 per cent sulfur. This sample was taken on bottom soil in virgin sod that received seepage water from surrounding higher land. These facts are mentioned to show that occasionally soils are found that have an abnormal amount of sulfur. The results from these three samples were not included in table 2.

In the humid sections, soils in alfalfa had 120 pounds less sulfur per acre than soils in virgin sod, while the soils cropped to grain had 180 pounds less. There were, apparently, losses of 17 and 25 per cent respectively, of the original sulfur-content.

In both the sub-humid and semi-arid sections, the soils cropped to alfalfa had more sulfur than the soils in virgin sod. The greatest difference was in the semi-arid section. This must mean that the deep-feeding alfalfa roots transferred large amounts of sulfur from the subsoil to the surface soil. Most of the organic matter of alfalfa roots is found in the surface foot of the soil. A large amount of alfalfa leaves are annually dropped on the soil where they

decay. Accumulation of organic matter, rich in protein material would increase the sulfur content of the surface soil. The average sulfur content of the soil cropped to grain was less than the sulfur content of the soils in virgin soil, but the loss of sulfur due to grain-cropping was small in the sub-humid and semi-arid sections.

In the humid section, the soils cropped to grain had 1000 pounds less nitrogen per acre than the soils in virgin sod, or a loss of about 26 per cent. The loss of nitrogen paralleled the loss of sulfur. The soils in alfalfa had less nitrogen than the soils in virgin sod, but the difference was only 8 per cent.

In the sub-humid section, the nitrogen content of the soils in alfalfa and the soils in virgin sod differed by only 200 pounds per acre, or only 5 per cent. Alfalfa, therefore, practically maintained the nitrogen content of the soil.

TABLE 4
Relative sulfur-content of crops and phosphorus based on averages of several published analyses

CROP	AVERAGE CROP YIELD	SULFUR IN CROP		PHOSPHORUS IN CROPS	
		<i>per cent</i>	<i>pounds</i>	<i>per cent</i>	<i>pounds</i>
Alfalfa hay	5,000 lbs.	0.287	14.25	0.24	12.00
Corn, grain	20 bu.	0.170	1.90	0.30	3.36
Corn, stover	200 lbs.	0.126	1.51	0.20	2.40
Total corn crop			3.41		5.76
Oats, grain	25 bu.	0.189	1.51	0.35	2.80
Oats, straw	1,260 lbs.	0.195	2.44	0.09	1.13
Total oats crop			3.95		3.93
Wheat, grain	15 bu.	0.170	1.53	0.37	3.33
Wheat, straw	1,250 lbs.	0.119	1.79	0.06	0.75
Total wheat crop			3.32		4.08

The soils in virgin sod has 980 pounds more nitrogen than the soils cropped to grain, or a loss of 23 per cent. This loss was much greater than the loss of sulfur for this section.

In the semi-arid section, the soils in alfalfa had more nitrogen than the soils in virgin sod, which shows that the alfalfa has taken enough nitrogen from the air not only to compensate for that removed in the hay, but to increase the nitrogen-content of the soil. These soils also increased in sulfur due, apparently to the transference of sulfur from the subsoil and the addition in rain. The soils cropped to grain had 560 pounds less nitrogen than the soils in virgin sod, a loss of 19 per cent while the sulfur loss was 11 per cent.

In the central and western part of the state apparently, the additions and losses of nitrogen compensated each other. In the eastern part of the state the losses were greater than the gains.

The changes in carbon-content almost paralleled the changes in nitrogen.

Changes in the organic-content of the soil naturally cause changes in the sulfur-content as well as in the nitrogen-content.

The soils in the humid and sub-humid sections contain a little over one and one-half times as much phosphorus as sulfur. In the semi-arid section, the phosphorus-content is nearly three times that of sulfur.

Table 4 presents the relative content of common farm crops of phosphorus and sulfur. Corn and wheat take more phosphorus from the soil than sulfur while oats take an equal amount of each element. Alfalfa requires more sulfur than phosphorus. Alfalfa removes more sulfur than any other crop. Table 3 shows that less sulfur has been lost from alfalfa fields than from those cropped to grain. This is explained by the transference of sulfur from the subsoil by the deep roots of alfalfa. Also the loss from leaching and washing is less in the field devoted to alfalfa.

Assuming that the average amount of sulfur removed annually by such crops as corn, wheat and oats is 3.5 pounds, the amount removed in 40 years would be 140 pounds. Table 3 shows that more than this amount has been lost from cultivated soils in eastern Kansas. This simply means that the sulfur added in rainfall is not sufficient to compensate for the amount of sulfur lost by leaching.

CONCLUSION

Whether sulfur is now a limiting element in crop production on Kansas soils will have to be determined by fertilizer tests in which sulfur is one of the leading elements. This study has shown that sulfur is decreasing in eastern Kansas soils and that this decrease is proportionate to the amount removed by crops. The amount added by rain is apparently not even sufficient to make good that lost by leaching. The loss of sulfur is greater than phosphorus and somewhat proportionate to the loss of nitrogen. These elements are known to be limiting elements in crop production. If sulfur continues to be lost from the soil in greater amounts than it is supplied it is reasonable to conclude that it also will be a limiting factor in crop production if it is not one already.

SUMMARY

1. In this paper are presented the amounts of sulfur found in 96 Kansas soils, some cropped to grain, some to alfalfa, and some in virgin sod.
2. The use of magnesium nitrate instead of sodium peroxide for determining sulfur in soil is described.
3. In the eastern or humid section of the state, the loss of sulfur in soils cropped to grain is proportionate to the loss in nitrogen. In the central and western parts of the state the loss is small, if any.
4. The amount of sulfur removed from the soil is apparently not compensated for by the amount added in rain. If sulfur continues to be removed faster than it is supplied, the possibility of sulfur being, or becoming, a limiting factor in crop production is indicated by this study.

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RELATION BETWEEN HEAT OF WETTING, MOISTURE EQUIVALENT AND UNFREE WATER

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INTRODUCTION

Water is one of the best indices of the physical characteristics of soil. Texture, structure, colloidal and organic contents, activation of surface, etc., tend to be revealed by the behavior of the soil toward water. Heat of wetting, moisture retentiveness, unfree water, etc., are mainly the resultant and expression of these characteristics.

The question of the relationship of these various soil-water manifestations to each other is obviously of much interest. If the relationship is of positive nature, then it is also of considerable practical importance, because in that case the simplest, most convenient and rapid method could be selected to obtain the other relationships and to make a comparative study of soils.

With these objects in view a comparative study was made of the relationship that exists between a number of soil-water manifestations, but principally those of heat of wetting, moisture-equivalent and unfree water.

METHODS AND PROCEDURE

The heat of wetting was determined according to the method described in a former publication (2).

It consisted of placing about 50 gm. of air-dry soil in a wide glass tube and allowing it to dry in an electrically heated oven at a temperature of 105°C. for about 24 hours. The tube was then taken out, closed with a rubber stopper and allowed to attain the room temperature. After the exact weight and temperature were ascertained, the soil was quickly and carefully poured into a calorimeter containing 100 gm. of water and the heat of wetting was ascertained. Extreme care was taken before mixing to have both the soil and water at exactly the same temperature and very nearly that of the room temperature. In order to be able to convert, if necessary, the rise of temperature into heat calories, the water-equivalent of the calorimeter was determined. This was found to be 25 gm. of water. The specific heat of soils was about .200 for the mineral soils and about .300 for the organic soils.

The unfree water was measured by means of the dilatometer method already described (1) with the modification of washing the soils free of soluble salts before their unfree water was measured. This was done in order to eliminate any effect of soluble salts on the unfree water. Two different moisture contents were employed for each soil, one was at about the optimum stage and the other at the proportion of 20 gm. of soil to 15 cc. of water. Before the final measurement was made, the soils were subjected to freezing and thawing once or twice. The soils were then placed at a temperature of -1.5°C. and allowed to come to equilibrium and the volume on the dilatometer stem recorded. They were then immersed at a temperature of about -12°C. for about fifteen minutes, and then returned to the original temperature.

After equilibrium was attained, the volume on the dilatometer stem was again read and from the two readings the unfree water could be calculated. The term unfree water is used in this paper to designate the soil water remaining unfrozen at the temperature of slightly below zero. All the water which freezes very readily at or very near zero is designated as free water. The free water is beyond the direct influences of the soil and freezes like water in mass, while the unfree water is under the direct influences of the soil and consequently is not at liberty to freeze as the free water.

For the moisture-equivalent the Briggs-McLane apparatus was used. The machine was regulated to run at a speed of about 2450 revolutions per minute and was allowed to run for 40 minutes. Thirty grams of the finer textured types of soil were used, 30 gm. of sand, and 10 to 15 gm. of the peats and muck.

RESULTS

The experimental results obtained on the heat of wetting, unfree water and moisture equivalent on a number of different types of soils, and the relationship that exists between these different soil-water manifestations, are shown in the adjoining table.

There is a rather close relationship in the various soils between the unfree water and the heat of wetting. The ratio ranges only from 0.0408 in soil 20 to 0.0675 in soil 1 with an average ratio of 0.05305 for all of the soils.

It would appear that there is a tendency for soils with much organic matter to give a smaller ratio than soils with very little or no organic matter. But the organic matter content, cannot be entirely responsible for this difference because peat and muck which represent the extreme organic soils have a ratio as large as the mineral soils with no or very little organic matter. A more satisfactory explanation for the difference seems to be found in the hypothesis that among soils causing about the same amount of water to become unfree, some possess a more activated surface, which results in more heat of wetting. Experimental results along this line will be presented in greater detail in a subsequent paper.

Considering next the ratio of moisture-equivalent and heat of wetting it is seen that there is not a very close relationship between these two factors in the various soils. The ratio ranges from 0.0953 in soil 20 to 0.3233 in soil 5 with an average ratio of 0.1685 for all of the soils. The ratio is quite close in many of the soils, but in others it varies considerably.

It is not surprising that there is no closer relationship between the heat of wetting and moisture-equivalent, because besides the naturally different specific properties of the various soils which would affect these two factors differently, the moisture-equivalent method does not give a true and an exact equivalent of moisture for all soils. In very fine textured and colloidal soils which allow the water to get out only with much difficulty and especially when compacted by centrifuging, the moisture-equivalent is considerably greater than it should be in comparison with that in coarse textured and porous soils. In some of the clay soils, for instance, the moisture-equivalent is as much as 25 per cent above what it should be. Results obtained by the

freezing point method indicate that with some soils the moisture-equivalent obtained is true but with others it is too high. When the moisture-equivalent, therefore, is not absolutely true in all of the different soils, its relationship with the heat of wetting would tend to vary accordingly. But even if the moisture-equivalent, were absolutely true in all the soils, probably its rela-

TABLE 1
Relationship between heat of wetting, unfree water and moisture-equivalent

SOIL NUMBER	SOIL TYPE	HEAT OF WETTING	UN-FREE WATER	MOISTURE EQUIVALENT	U.W.* H.W.	M.E.† H.W.	M.E.‡ U.W.
		°C.	per cent	per cent			
1	Sand.....	0.2	1.35	3.8	0.0675	0.1900	2.815
2	California Handford sandy loam.....	0.8	5.2	15.0	0.0650	0.1875	2.885
3	Ohio silt loam.....	0.7	4.6	21.8	0.0657	0.3114	4.740
4	Ohio silt loam.....	0.7	3.6	21.3	0.0437	0.3043	5.917
5	Cornell silt loam.....	0.9	5.25	29.1	0.0583	0.3233	5.541
6	Pennsylvania silt loam.....	0.9	5.0	28.9	0.0555	0.3211	5.780
7	Pennsylvania silt loam.....	1.1	7.0	29.2	0.0636	0.2655	5.600
8	Rhode Island sandy loam.....	1.1	6.0	21.7	0.0545	0.1972	3.617
9	Michigan silt loam.....	1.65	10.20	25.5	0.0618	0.1546	2.500
10	Wisconsin Superior clay.....	1.95	10.75	28.2	0.0548	0.1446	2.635
11	California Capay clay.....	2.0	11.1	33.5	0.0555	0.1675	3.020
12	Michigan clay loam.....	2.2	13.6	30.2	0.0618	0.1373	2.221
13	California Willows clay.....	2.2	12.9	32.2	0.0586	0.1464	2.496
14	Minnesota Clyde silt loam.....	2.55	11.0	29.8	0.0431	0.1170	2.710
15	Minnesota black surf.....	2.85	14.0	36.5	0.0491	0.1281	2.607
16	Illinois clay loam.....	2.9	15.2	36.3	0.0524	0.1252	2.338
17	Minnesota Fargo clay.....	3.0	16.0	39.0	0.0533	0.1300	2.438
18	Iowa Carrington clay loam.....	3.4	16.0	36.5	0.0472	0.1074	2.282
19	Illinois clay loam.....	3.5	14.3	35.8	0.0408	0.1023	2.504
20	Illinois black clay loam.....	3.5	14.7	34.0	0.0420	0.0971	2.313
21	California Merced clay.....	4.0	17.8	40.2	0.0445	0.1005	2.258
22	California Stockton clay adobe.....	4.1	18.7	49.5	0.0456	0.1207	2.647
23	Wisconsin black clay loam.....	4.9	20.9	46.7	0.0426	0.0953	2.235
24	Muck.....	8.0	37.0	95.5	0.0462	0.1194	2.581
25	Muck.....	12.6	67.0	147.0	0.0532	0.1167	2.194

* Percentage of unfree water divided by rise of temperature of 50 gm. of soil in 100 gm. of water.

† Moisture-equivalent divided by rise of temperature of 50 gm. of soil in 100 gm. of water.

‡ Moisture-equivalent divided by percentage of unfree water.

tionship with the heat of wetting would not be exceedingly close in the various soils, because the two factors are affected differently by soil properties and conditions.

It was originally thought that there might be a rather close relationship between the heat of wetting and moisture-equivalent since both factors were considered to be controlled principally by three properties, namely, texture, colloidal and organic matter content. It is evident from the experimental

results obtained, however, that there is at least one more property coming into play which affects the results appreciably, namely the activation or nature of the surface.

The ratio between the unfree water and the moisture-equivalent shows that there is not a very close and consistent relationship between the two factors. The ratio ranges from 2.194 in soil 25 to 5.917 in soil 4 with an average ratio of 3.1456.

Comparison of the three ratios with one another shows, also, that there is no very close and consistent parallelism or relation between any two ratios.

SUMMARY

In this paper are presented the results of an investigation conducted to ascertain the relationships that exist between heat of wetting, unfree water, and moisture-equivalent of soils. The experimental results show that there is a close and consistent relationship between the heat of wetting and the unfree water, but there appears to be no close and regular relationship between the heat of wetting and moisture-equivalent or between unfree water and moisture-equivalent.

Evidences obtained go to indicate that the moisture-equivalent method does not give a true and absolutely equivalent moisture in all of the various soils. Some of the fine textured and colloidal soils contain considerably more moisture than their true moisture-equivalent.

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SULFUR AS A FERTILIZER FOR COTTON

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In 1920 the Texas Agricultural Experiment Station conducted at Substation No. 5, Temple, Texas, preliminary experiments¹ with sulfur to determine its value as a fertilizer for cotton and also to ascertain whether it would be possible to control or prevent the Texas root-rot of cotton, *Phymatotrichum omnivorum* (Shear) Duggar by the use of sulfur. Acid phosphate alone and in combination with sulfur was included in part of this work, although, in previous experiments with fertilizers, phosphatic materials had never given large increases in yield.

This preliminary work was conducted on Abilene clay soil, which is generally considered as not needing lime or phosphorus. This soil has been cropped continuously to cotton for the last ten years.

The plots were one-twentieth of an acre in size, with rows 36 inches apart. The fertilizer materials were applied in the drill on March 27, and the cotton was planted on April 10.

Table 1 gives the plan of the experiment, and the yield of lint and seed cotton to the acre.

It will be noted that sulfur applied at the rate of 1000 pounds to the acre made a much larger yield than any other treatment. Sulfur in combination with acid phosphate produced larger yields than acid phosphate alone. Sulfur, however, at the rate of 500 pounds to the acre, on plot 4, produced a smaller yield than did any other treatment in the test. The average yield of the three plots which received sulfur was 374.74 pounds of lint cotton to the acre; of the plots which received sulfur and phosphorus, 359.08 pounds; of the plot which received phosphorus alone, 259.76 pounds; and the average yield of the soil checks was 276.63 pounds to the acre. In general, sulfur increased the yield, while acid phosphate did not.

The cotton on all plots made comparatively small vegetative growth, but the plants on plot 2 were, as an average, about 3 or 4 inches taller than the plants on any other plot in the experiment. The average height of the plants on the plots which were treated with sulfur was 2-3 inches greater than the height of the plants on the plots which did not receive sulfur. The foliage on plot 2 was darker green than the foliage on the other plots.

Texas root-rot² was present uniformly on all plots, and by actual count affected 90-95 per cent of the plants. The plants on plot 2, however, were not affected so early as the plants on the other plots. This fact probably explains the great increase in yield of plot 2 over the yields of the other plots.

In 1920, in another experiment with sulfur as a fertilizer for cotton on a shallow phase of Houston clay soil on the farm of S. H. Cater near Substation No. 5, sulfur was applied alone at the rates of 100, 300, and 600 pounds to the acre in the drill, on March 25, and the cotton was planted on April 8. The size of the plots was one-eighth of an acre. The results of this experiment are given in table 2.

¹ Credit is due Mr. D. T. Killough, Superintendent, for conducting the field work and collecting the yield data reported in this paper.

² The root-rot investigations are being conducted by Dr. J. J. Taubenhaus, Chief, Division of Plant Pathology and Physiology. The authors are indebted to him for the notes on root-rot reported in this paper.

Table 2 shows that the yields of cotton increased with the amount of sulfur applied, although the increase in yield was not directly proportional to the increase in the amount of sulfur used.

Apparently, sulfur had no effect on the root-rot for the disease was uniformly distributed on all the plots, irrespective of the treatments. Very little difference in growth of the cotton could be noted on the various plots.

TABLE 1

Yield per acre of lint and seed cotton on plots in experiment with sulfur and acid phosphate, 1920

PLOT NUMBER	TREATMENT		YIELD PER ACRE	
	Material	Amount	Lint	Seed cotton
		<i>lbs.</i>	<i>lbs.</i>	<i>lbs.</i>
1	Nothing-check		275.04	720.00
2	Sulfur	1000	527.35	1380.50
3	Sulfur	500		
	Acid phosphate	400	368.63	965.00
4	Sulfur	500	234.93	615.00
5	Nothing-check		295.09	772.50
6	Sulfur	50		
	Acid phosphate	400	349.53	915.00
7	Acid phosphate	400	259.76	680.00
8	Sulfur	50	361.94	947.50
9	Nothing-check		259.76	680.00
Average of checks			276.63	724.16

TABLE 2

Yield per acre of lint and seed cotton in the sulfur fertilizer experiment, 1920

PLOT NUMBER	TREATMENT	YIELD PER ACRE	
		Seed cotton	Lint
		<i>lbs.</i>	<i>lbs.</i>
1	Nothing-check	496	187.43
2	100 pounds sulfur	608	229.76
3	300 pounds sulfur	632	238.83
4	600 pounds sulfur	776	293.25

The results in 1920, presented in tables 1 and 2, suggested that it would be advisable to establish a more comprehensive experiment with sulfur with the idea of securing additional information on its use as a fertilizer and its effect on the soil, such as the lime requirement, hydrogen-ion concentration, and bacterial activities.

Accordingly, an experiment was outlined, which comprised applications of sulfur and lime, alone and in combination, in amounts varying from 500 pounds to 10,000 pounds to the acre. This work is conducted on Simmons clay and Abilene clay at Substation No. 5, Temple, Texas. These soils are classified as high terrace soils by the Bureau of Soils, and are, as a rule, well supplied

with lime and phosphorus. The hydrogen-ion concentration of the Abilene clay soil was pH 8.00 at the beginning of the experiment.

Two series of 16 plots each are included in the experiment. Series 1 comprises a three-year rotation of corn, small grain (wheat or oats) followed by cowpeas as a catch crop, and cotton. Series 2 receives the same treatment as series 1, but it includes continuous cotton instead of cotton in rotation. Series 2 is conducted on soil which has grown cotton for the last eleven years. In series 1, the treatments are applied to the cotton only; thus each of the three acres in the rotation will receive sulfur only once in three years, while in series 2 the land receives the treatments every year. The size of plots in the rotation, series 1, is one-twenty-second of an acre and in series 2, one-forty-fourth acre.

Lime and the sulfur were applied broadcast on April 20, 1921 and the cotton was planted on May 4. Inoculated sulfur and pure hydrated lime were used in this experiment.

TABLE 3
Germination of cotton planted in soil treated with sulfur, and hydrogen-ion concentration of the soil on April 1, 1921

BOX NUMBER	SULFUR APPLIED PER ACRE	GERMINATION			REACTION APRIL 1
		First	Second	Third	
		<i>per cent</i>			<i>pH</i>
1	Nothing-check	76	Normal	Normal	7.00
2	500 pounds	84	A few	None	
3	1000 pounds	82	A few	None	
4	2500 pounds	66	None	None	2.20
5	5000 pounds	70	None	None	2.20
6	10000 pounds	76	None	None	2.20

In outlining this experiment it was thought that the heavy applications of sulfur would retard and perhaps prevent the normal development of the cotton plant in the field, which condition, in this case, was desired.

In order to obtain some information on this point some tentative tests were made in the laboratory. In these tests sulfur was applied at the rates of 500, 1000, 2500, 5000, and 10000 pounds to the acre on sandy loam soil, in wooden germinating boxes, on February 19. The boxes were 18 inches long, 8 inches wide, and 4 inches deep. The results of this test are presented in table 3.

Germination was normal in all boxes in the first planting. Cotton seeds were planted again in the same boxes on March 14, 1921. The seeds in box 1, check, germinated normally by March 19 and a few seeds germinated in boxes 2 and 3. There was no germination in the other boxes. The germination test was repeated again on March 23 and the seeds on the check germinated in a normal manner while no germination occurred in the other boxes. These results are in accord with those of Shedd (3).

The hydrogen-ion concentration of the soil with the three heaviest applications in the boxes was determined tentatively on April 1, 1921 by the method suggested by Wherry (4). The results of this determination are shown in the last column of table 3. It should be kept in mind that the soil used in this laboratory test was entirely different from the soil on which the field experiment was conducted.

Unfortunately the laboratory work planned in connection with the field work could not be carried out, and the yield data are presented for what they may be worth.

The amounts of lime and sulfur applied to the acre and the yields of both series of plots are given in table 4.

It will be noted from table 4 that the highest yield of both lint and seed cotton in the rotation, series 1, resulted from the application of 5000 pounds of lime. The lowest yield in series 1 was obtained from the plot which received

TABLE 4
Yields per acre of lint and seed cotton in experiment with lime and sulfur, 1921

PLOT NUMBER	TREATMENT	ROOT-ROT		SEED COTTON		LINT	
		Series 1 (Rotated)	Series 2 (Not rotated)	Series 1 (Rotated)	Series 2 (Not rotated)	Series 1 (Rotated)	Series 2 (Not rotated)
		per cent	per cent	lbs.	lbs.	lbs.	lbs.
1	Nothing-check.....	10	8	858.00	792.00	290.54	271.10
2	500 pounds lime.....	6	10	864.00	660.00	291.60	225.09
3	500 pounds sulfur.....	2	12	800.00	550.00	271.47	187.37
4	500 pounds lime and 500 pounds sulfur.....	4	25	880.00	418.00	299.59	143.02
5	2500 pounds lime.....	3	35	887.50	396.00	300.66	136.62
6	Nothing-check.....	2	50	880.00	374.00	300.04	129.03
7	2500 pounds sulfur.....	4	95	704.00	27.50	240.38	9.48
8	2500 pounds lime and 2500 pounds sulfur.....	3	92	770.00	24.05	262.92	8.29
9	5000 pounds lime.....	0	86	902.00	108.62	306.68	37.47
10	5000 pounds sulfur.....	1	78	704.00	110.00	239.79	37.71
11	Nothing-check.....	5	70.5	748.00	286.00	254.38	97.00
12	5000 pounds lime and 5000 pounds sulfur.....	4	45	671.00	275.00	229.24	92.73
13	10000 pounds lime.....	T	18	814.00	396.00	277.38	132.58
14	10000 pounds sulfur.....	T	8.5	638.00	396.00	217.50	133.53
15	10000 pounds lime and 10000 pounds sulfur.....	T	5	792.00	484.00	270.03	162.23
16	Nothing-check.....	5	4	682.00	594.00	232.91	198.52
Average of checks.....				792.00	511.50	269.46	173.91

the largest amount of sulfur, 10,000 pounds. In every case, with one exception, the plots which received sulfur alone produced smaller yields than did the corresponding plots which received both lime and sulfur in equal quantities.

This is probably due to the fact that the acidity which was formed by the oxidation of sulfur was injurious to the cotton plant, and consequently reduced the yield. The lime which was applied with the sulfur neutralized some of the acid which developed, and as a result, improved the soil conditions, which resulted in an increase in yield as compared with the corresponding plots

which received sulfur alone. Lipman, Prince, and Blair (2) found that applications of 1000, 2000 and 4000 pounds of sulfur made sharp increases in the hydrogen-ion concentration and lime requirement of the soil after the sulfur had been in the soil about six weeks. In our experiment it seems quite probable that similar increases in the hydrogen-ion concentration and lime requirement occurred, and that the lime which was present in the soil neutralized part of the acidity and consequently increased the yields as mentioned above.

Quite different results were secured with the non-rotated cotton, series 2. There is apparently no correlation between the yield of cotton and the quantities of lime and sulfur applied. This result is due, partially at least, to the irregular distribution of root-rot on this series. If the percentages of root-rot on series 1 and series 2 are compared, it seems that the irregular distribution of root-rot overshadowed the influence of the fertilizer treatments on series 2. Previous experiments (1, p. 12-13) with rotations and continuous culture of cotton at Substation No. 5 show that the root-rot of cotton may be controlled to a great extent by the proper use of rotations and cultural methods.

While the tests in the laboratory showed that the heavy applications of sulfur formed enough acid to prevent germination and growth, no apparent injury resulted from the heavy applications in the field. The soil on the plots, as stated previously, is fairly well supplied with lime, having a pH value of 8.00 at the beginning of the experiment. The foliage on the plots which received 5000 and 10000 pounds of sulfur alone was lighter green in color than the foliage on the other plots.

Shedd (3) found that 5000 to 7000 pounds of sulfur to the acre killed cabbage, mustard, and radishes, but the soil he used was slightly acid.

Lipman, Prince, and Blair (2) applied sulfur to the soil at the rates of 200, 500, 1000, 2000 and 4000 pounds to the acre to barley and soybeans (as a residual crop after the barley). The barley germinated fairly well on all the plots, but there was evidence of injury with 1000 pounds of sulfur. Practically all of the plants on the plots that received 4000 pounds had been killed before harvest. One thousand pounds of sulfur depressed the germination of soy beans, and there were very few plants on the plots that received 2000 and 4000 pounds of sulfur.

SUMMARY

In 1920 cotton was grown on plots to which sulfur was applied at the rates of 50, 500, and 1000 pounds to the acre. Acid phosphate was also applied alone and in combination with sulfur at the rates of 400 pounds to the acre.

In general, sulfur increased the yield of cotton. The average yield of the three plots which received sulfur alone was 374.74 pounds lint cotton to the acre; of the plots which received acid phosphate and sulfur, 359.08 pounds; of the plot which received acid phosphate alone, 259.76 pounds; while the average yield of the soil checks was 276.63 pounds to the acre. The cotton on the plot which received 1000 pounds sulfur made a larger vegetative growth and had darker green leaves than the cotton on the other plots.

Another experiment was conducted in 1920 in which sulfur was applied to the soil at the rates of 100, 300, and 600 pounds to the acre. The yields of cotton increased with the increase in the amount of sulfur applied.

The field work in 1921 included applications of sulfur and lime, alone and in combination, in amounts varying from 500 to 10,000 pounds to the acre. In this experiment the largest yield of cotton resulted from the plot which received 5000 pounds of lime. Apparently the sulfur alone had a tendency to depress the yield. Where lime was applied with the sulfur, in the rotated series, the yield of cotton was larger than the yields of the plots which received corresponding amounts of sulfur. Apparently lime mitigated the injurious effect of the heavy applications of sulfur by neutralizing the acid formed by the oxidation of the sulfur.

In these experiments sulfur has neither prevented nor controlled the development of root-rot of cotton.

This work with some modification is being continued.

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ADSORPTION OF PLANT FOOD BY COLLOIDAL SILICA

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INTRODUCTION

The question as to the exact manner in which soil colloids retain plant food, whether by chemical reaction or by physical adsorption, is one that has never been settled.

Biederman (1) was one of the first to emphasize the importance of absorption by soils. He felt that the ability of the soil to take up soluble fertilizers and then give them back to the soil was of great significance to practical agriculture as well as extremely interesting from a philosophical point of view. One question he raised was "In what form do plants obtain their nourishment from the soil?"

Salomon (7) determined the adsorption of calcium by various soils and soil constituents and among the latter was hydrated silicic acid. He added enough ammonia to neutralize the nitric acid of the calcium nitrate. Under these conditions he found that the adsorption of the calcium by the hydrated silicic acid increased with the concentration of the calcium nitrate.

Frey (2) pointed out that it is very probable that the absorptive power of soil is proportional to the silicate bases such as alumina, ferric oxide, etc. He worked to a large extent with soils as a whole and hence his problem became very complex.

Van Bemmelen (8) stated that the soil contains colloids such as iron oxide, silicic acid and humus. He believed that these colloids absorbed some of the salts from solution, but how this was accomplished he was uncertain.

Some more recent workers (6) have shown more plainly that there are definite soil colloids and have succeeded in mechanically separating a large part of the colloidal material from soils. The composition of these colloids vary with different soils.

In most of the work thus far done on soil colloids, the systems with which investigators have worked have been so complex that it has not been possible to say whether one had physical absorption or chemical combination. A preliminary paper by Gordon and Starkey (4) contrasts the different soil colloids in their power of absorbing salts and in making them available for plant food. The purpose of the present investigation was to further study absorption by silica in order to find what power colloidal silica had of holding the salts commonly used in plant nutrition work. Determinations were made for the adsorption of both negative and positive ions to find out just how much, if any, splitting of the molecule occurred during adsorption.

MATERIAL

Since the authors believed that the soil colloids at times existed as a hydrogel and at other times as a hydrosol (3), it was decided to try the adsorption of the salts by both the hydrogel and hydrosol of silica.

Silica gel was bought of the Davidson Chemical Company, and was washed with distilled water until the wash water gave no test for sulfates. When the

washing was finished and the hydrogel drained, it contained 25-35 per cent water. The exact water-content was determined on each portion used. The hydrosol was made in the usual way by treating sodium silicate with dilute hydrochloric acid and dialyzing until free from electrolytes. In this way a very stable hydrosol was obtained.

The salts used in the work were potassium nitrate, potassium sulfate, potassium acid sulfate and the calcium and magnesium salts of the same acids. These salts were recommended by the National Research Council and put out by J. T. Baker and Company.

EXPERIMENTAL WORK

One-gram portions of the hydrogel were weighed into sixty glass stoppered bottles of about 400-cc. capacity. While the hydrogel was being weighed into bottles, a sample of it was taken for moisture determination. Two 100-cc. portions of the solutions of the salts to be tested were added to the bottles containing the gel and the bottles were sealed and shaken until equilibrium was established.

In the analytical work the greatest precautions were taken to insure the most accurate and comparable results. A brief outline of the analytical work is given.

A portion of the solution which was to be analyzed for nitrogen was measured with a pipette into a flask. Five grams of iron, 200 cc. of water and 10 cc. of concentrated sulfuric acid were added to the flask, and the mixture carefully heated for thirty minutes. It was then cooled and the nitrogen distilled over as in the Kjeldahl method.

Sulfur was determined in the usual way; that is, by precipitating as barium sulphate, igniting and weighing.

Phosphorus was determined by the volumetric method as adopted by the Association of Official Agricultural Chemists.

Potassium was determined by the method of the Association of Official Agricultural Chemists.

Calcium was precipitated as calcium oxalate. After washing the precipitate it was dissolved in sulfuric acid and titrated against potassium permanganate in the usual way. One cubic centimeter of the potassium permanganate solution equalled 0.0001 gm. calcium.

Magnesium was determined by precipitating it as magnesium ammonium phosphate, washing the precipitate carefully with dilute ammonium hydroxide solution, drying it on the filter paper at 60°C., dissolving it in a known amount of standard acid and titrating back with standard alkali. One cubic centimeter of the standard acid was equal to 0.0001 gm. magnesium. All determinations were run in duplicate and each result reported is the average of the two.

EXPERIMENTAL RESULTS

Table 1 shows the data obtained in testing the adsorption of the hydrogel from solutions of potassium, calcium and magnesium nitrate and potassium, calcium and magnesium sulfate.

The table shows some results that were anticipated as well as some that were not. One would expect positive adsorption in wet gel. Columns 5 and 10 show such results. In the dry gels there was negative adsorption except in

the case of magnesium and the sulfate of potassium. The negative adsorption might indicate one of three things: (a) that all the water of hydration did not act as a diluent; (b) that the water was more strongly adsorbed than the salt, or (c) that we have fine capillaries with which to deal, and, if so, according to Mathieu (5) the solution would be more concentrated due to the solution in the capillaries being less concentrated. The ions of the respective salts are absorbed in practically equivalent quantities with the exception of potassium sulfate.

In table 2 the results are given where acid phosphate solutions of calcium, magnesium and potassium of varying concentrations are used with the hydrogel of silica. The columns are arranged similarly to those in table 1.

In table 2 as in table 1 we find positive adsorption with the wet gel, but with the gel when the water of hydration is figured as water of dilution the phosphates act somewhat in contrast to the nitrates and sulfates. In general the cation is negatively absorbed, while the anion is positively absorbed. This would seem to point to a splitting of the molecule were it not for the fact that we are dealing with a fraction of a milligram. The higher concentrations of calcium acid phosphate give negative adsorption for both ions. This may have been caused by a slight precipitation as a saturated solution was used.

Table 3 gives us the results obtained when the phosphates of calcium, magnesium and potassium were used with the hydrosol of silica. This hydrosol contained 3.1 per cent of silica. The columns are arranged similarly to those in the preceding tables.

Column 6 shows that the metals are always negatively adsorbed except in one case, and in general the negative adsorption is quite great. The phosphat ion, too, is negatively adsorbed in all but two cases, but the negative adsorption is not so great as that of the positive ion. This would indicate the same tendency in the splitting of the molecule as in the case of the hydrogel.

The tendency by which phosphates are held by the hydrogel is also interesting. Two samples of the hydrogel were shaken with 0.05 N solutions of potassium acid phosphate and calcium acid phosphate, respectively. All the solution was then drained from the gel which was placed on a filter and washed repeatedly with distilled water. The water was poured on the gel in 50-cc. portions and the gel allowed to drain thoroughly after each addition. Fifty-cubic centimeter portions of the washings were analyzed for phosphorus at intervals. Results are given in table 4.

It will be seen from table 4 that the salt that was held by the silica was remarkably resistant to washing. Also, that the portion of water obtained by washing after the gel stood overnight in a moist condition on the filter is much richer in phosphorus than washings obtained from the gel which stood for only a few minutes in a moist condition. A sample of the hydrogel which had been shaken with magnesium acid phosphate solution was washed six times a day for thirty days with 50-cc. portions of distilled water and at the end of that time still contained phosphate. The sample of hydrogel, washed

TABLE 1
Adsorption of nitrates and sulfates by the hydrosol of silica

1	2	3*	4	5	6	7	8	9	10	11
SOLUTIONS USED	METAL IN 20 CC. OF ORIGINAL SOLUTION	METAL IN 20 CC. OF SOLUTION AFTER DILUTION	METAL FOUND IN 20 CC. AFTER SHAKING	METAL ADSORBED PER GM. OF WET GEL	METAL ADSORBED PER GM. OF DRY GEL	ANTION IN 20 CC. OF ORIGINAL SOLUTION	ANTION IN 20 CC. AFTER DILUTION	ANTION IN 20 CC. AFTER SHAKING	ANTION ADSORBED PER GM. OF WET GEL	ANTION ADSORBED PER GM. OF DRY GEL
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
KNO ₃	18.096	15.994	16.048	0.20	-0.07	6.027	5.197	5.595	0.04	-0.06
Ca(NO ₃) ₂	12.420	11.660	12.290	0.02	-0.16	7.630	7.160	7.742	0.02	-0.15
Mg(NO ₃) ₂	12.510	11.530	10.931	0.24	0.02	14.552	13.410	12.530	0.03	0.29
K ₂ SO ₄	39.248	31.420	36.480	0.42	-0.12	16.528	15.160	15.012	0.43	0.31
CaSO ₄	3.880	3.543	3.665	0.03	-0.03	4.076	3.722	4.002	0.01	-0.06
CaSO ₄	12.888	11.770	12.127	0.10	-0.09	9.066	8.260	8.919	0.02	-0.01
MgSO ₄	12.349	11.428	11.205	0.17	0.07	15.910	14.666	15.189	0.01	-0.01

* The third column shows the theoretical weight of metal in 20 cc. of the solution provided all the water in the gel acted as a diluent. The fourth column indicates milligrams of metal actually found in 20 cc. after adsorption was completed. The fifth column gives the milligrams of metal adsorbed per gram of gel if water contained in the gel is not figured as diluting the solution upon which the adsorption test is made. The sixth column shows milligrams of metal adsorbed if the water contained in the gel is figured as diluting the solution from which the adsorption test is made. From what has been said of the first six columns, columns seven to eleven, inclusive, should be clear.

TABLE 2
Adsorption of phosphates by hydrotel of silica

1	2	3	4	5	6	7	8	9	10	11
SOLUTIONS USED	METAL IN 10 CC. OF ORIGINAL SOLUTION	METAL IN 10 CC. AFTER DILUTION	METAL IN 10 CC. AFTER SHAKING	METAL TAKEN UP PER GM. WET GEL	METAL ADSORBED PER GM. DRY GEL	PO ₄ IN 10 CC. OF ORIGINAL SOLUTION	PO ₄ IN 10 CC. AFTER DILUTION	PO ₄ IN 10 CC. AFTER SHAKING	PO ₄ TAKEN UP PER GM. WET GEL	PO ₄ TAKEN UP PER GM. DRY GEL
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Ca(H ₂ PO ₄) ₂	5.468*	4.510	5.070	1.19	-1.07	9.252	7.792	8.998	0.77	-2.29
	5.432*	4.837	5.040	-0.43	9.252	8.243	8.998	0.47	-1.60
	5.367	4.868	4.923	0.19	-0.01	9.602	8.700	8.505	0.32	0.06
	2.599	2.235	2.429	0.05	-0.03	4.917	4.456	4.370	0.16	0.03
	2.689†	2.436	2.282	0.06	0.03	4.012	4.451	4.385	0.07	0.01
	0.847‡	0.768	0.664	0.09	0.02	1.876	1.700	1.264	0.05	0.05
	5.740	5.140	5.740	0.00	-0.12	10.334	9.352	8.928	0.43	0.08
	2.820	2.520	3.164	-0.05	-0.12	5.246	4.747	4.536	0.22	0.04
	1.456	1.317	1.876	-0.06	-0.11	2.696	2.240	1.922	0.23	0.10
	5.140	4.960	5.140	0.00	-0.06	14.217	13.730	13.808	0.12	-0.01
Mg(H ₂ PO ₄) ₂	2.700	2.670	2.680	0.00	-0.00	7.497	7.243	7.229	0.22	0.00
	1.350	1.300	1.200	0.04	0.03	3.532	3.413	3.313	0.06	0.03
KH ₂ PO ₄	12.600	11.500	11.200	2.15	0.05	10.545	9.810	8.783	0.27	0.25
	6.456	5.819	5.840	0.09	-0.01	5.270	4.890	4.003	0.19	0.25
	3.420	3.120	3.230	0.02	-0.20	2.830	2.630	2.091	0.11	0.13

* Amount in 1 cc.
† Amount in 20 cc.
‡ Amount in 50 cc.

TABLE 3
Adsorption of phosphates by hydrosol of silica

1	2	3	4	5	6	7	8	9	10	11
SOLUTIONS USED	METAL IN 10 CC. OF ORIGINAL SOLUTION	METAL IN 10 CC. AFTER DILUTION	METAL IN 10 CC. AFTER SHAKING	METAL ADSORBED PER GM. WET GEL	METAL ADSORBED PER GM. DRY GEL	PO ₄ IN 10 CC. OF ORIGINAL SOLUTION	PO ₄ IN 10 CC. AFTER DILUTION	PO ₄ IN 10 CC. AFTER SHAKING	PO ₄ ADSORBED PER GM. WET GEL	PO ₄ ADSORBED PER GM. DRY GEL
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Ca(H ₂ PO ₄) ₂	5.740	3.860	3.920	23.00	-0.65	10.334	6.950	7.171	45.00	-2.35
	2.820	1.900	2.128	9.90	-2.42	5.236	3.357	3.567	23.90	-2.23
	1.456	0.979	1.494	-0.04	-5.36	2.698	1.815	1.922	11.05	-1.18
Mg(H ₂ PO ₄) ₂	5.140	3.470	4.130	14.40	-12.41	14.217	11.436	10.632	51.20	8.49
	2.700	2.252	2.350	2.25	-4.97	7.970	6.031	6.110	26.60	-0.84
	1.350	1.126	1.356	0.08	-4.08	3.532	2.841	2.820	10.17	0.21
KH ₂ PO ₄	12.680	8.060	8.760	56.06	-7.00	10.545	7.096	7.102	49.20	-0.06
	6.556	4.344	4.144	33.03	2.97	5.272	3.548	3.624	23.83	-0.80
	3.416	2.229	2.424	14.03	-1.32	2.830	1.904	2.375	6.50	-4.00

as described above, was placed in a funnel of such size that the 50-cc. portion of wash water covered it about 2 cm. deep. It is evident that a very great amount of rainfall would be necessary to duplicate the amount of washing given this sample, and that it would take a very great time for ordinary rainfall to wash the sample free from the salt.

TABLE 4
Phosphates found in 50-cc. portions of wash water

	PO ₄ LEACHED FROM 1 GM. GEL.	
	Calcium acid phosphate solution	Potassium acid phosphate solution
	mgm.	mgm.
First washing.....	4.15	4.74
Eleventh washing.....	0.10	0.14
Twenty first washing.....	0.07	0.13
Thirty first washing.....	0.06	0.12
Forty first washing.....	0.06	0.12
Forty second washing after standing over night.....	1.31	0.54

DISCUSSION

In reviewing the tables it must be remembered that we are dealing with milligrams in our work, and since the adsorptions are in general small and sometimes only .01 mgm., a slight error would change the order of the adsorption. On the other hand, the results seem to point to the fact that the metals and also the nitrates and sulfate radicals have a tendency to be negatively adsorbed, or not adsorbed at all by colloidal silica, while the phosphate radical tends to suffer a slight positive adsorption in the case of the hydrogel.

SUMMARY

1. The metals are, in general, negatively adsorbed by both the hydrogel and the hydrosol.
2. The nitrate and sulfate radicals of calcium, magnesium and potassium suffer, in general, a slight negative adsorption.
3. The phosphate radical was positively adsorbed by the hydrogel and negatively adsorbed by the hydrosol.
4. In the case of the hydrogel, the phosphate radical was adsorbed to a greater degree than the radical with which it was associated.
5. A phosphate adsorbed in the hydrogel can be washed only with extreme difficulty.

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INFLUENCE OF HYDROGEN-ION CONCENTRATION ON THE ADSORPTION OF PLANT FOOD BY SOIL COLLOIDS

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INTRODUCTION

All know, according to Faraday's researches, that charges accumulate at the surface and, hence, at a boundary between two liquids, two solids, or liquid and solid, and that even between a gas and liquid we have a seat of electrical energy. Some believe that this seat has an electronic origin, while others believe that it is due to the Helmholtz's "double layer." In view of the latter, the charge on the surface of a solid or liquid would imply the existence of an equal and opposite charge on the other side of the interface, for a charge of a particular sign cannot exist without one of opposite sign in its proximity.

Perrin (6) has shown beyond the shadow of a doubt that the electrical charge on surfaces is in reality diminished, neutralized, or even reversed by ions of opposite charge. Acids and alkalies are very active in this power of conferring the electrical charges on surfaces. This is attributed to the fact that the H and OH ions are more readily absorbed than other ions, and, hence, the charge on an absorbent would depend on which of these ions is in excess.

Knud Estrup (4) has done some work along the line just mentioned. He showed that both the cation and anion were absorbed by charcoal in equivalent quantities when he worked with neutral salt solutions, and when the ions were not absorbed in equivalent quantities he attributed the difference to the presence of impurities in the form of acids and bases. In other work (3) with blood charcoal Estrup finds that the negative ion is positively absorbed in acid solutions, but on addition of a base the absorption of the negative ion disappears, giving place to the absorption of the positive ion.

It has been further shown that electrical charges tend to reduce surface tension. V. Boumat (1) finds that acids decrease surface tension and ascribes the fact to the accumulation of H-ions in the surface layer.

From the point of energetics, the following view of electrical adsorption has been formulated: Any process that will reduce the electrical energy at a surface will tend to take place, hence, in the case of a negatively charged surface, we will have positive charges collecting upon it in order to annul its charge, and vice versa. These bodies may be ions or colloidal aggregates.

A great many more or less obscure phenomena in the field of soil chemistry as well as in many other fields have been attributed to adsorption where it was not known whether they had physical adsorption, chemical reaction or an adsorption phenomena which might be attributed to charged aggregates where the adsorbing aggregate had a sign opposite to that of adsorbed substance.

Since it was known that OH and H ions were the most strongly adsorbed ions, and their adsorption should help to determine the charge on the colloid, it became of interest to determine the adsorption by single soil colloids under varying hydrogen-ion concentrations. It was hoped that such work might throw some definite light on how acidity or alkalinity of soil effects adsorption of plant foods, and also the availability of soil salts. A few of the results have already been published in a preliminary paper by Gordon and Starkey (5).

MATERIAL

The salts used were those prepared by the J. T. Baker Company and recommended by the National Research Council. They were the acid phosphates, sulfates and nitrates of potassium and calcium.

The hydrogels used were the hydrogels of silica and iron. The hydrogel of silica was obtained from the Davison Chemical Company. This hydrogel was washed free from all impurities before being used. The hydrogel of iron was prepared in our laboratory by precipitation from ferric chloride solution with ammonium hydroxide and washed free of ammonia and chlorides.

The apparatus used for determining the pH values of the solutions was of the Leeds, Northrup type. The Bailey hydrogen electrode was used in conjunction with a 1 *N* solution of KCl and the calomel electrode as described by Clarke (2). The mixtures of solutions and gels were shaken in 250-cc. ground-glass stoppered bottles with a mechanical shaker making about three oscillations per second.

TABLE 1
Adsorption of KH_2PO_4 by hydrogel of silica

NUMBER OF SOLUTION	REACTION	K ADSORBED PER GRAM GEL		PO ₄ ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	mgm.	mgm.	mgm.	mgm.
1	9.501	9.63	9.99	-4.15	-0.57
2	7.692	6.56	7.40	-4.15	-0.57
3	6.086	1.74	2.18	-3.15	0.00
4	3.888	-0.68	0.71	-1.36	1.92

EXPERIMENTAL WORK

A series of four or five solutions of each of the salts used was made up. The pH values in each series were varied by adding the required amounts of NaOH or HCl (ranging from neutral to N/5) before the solutions were made up to the mark. The salt concentration was kept constant throughout. The concentration of each solution was determined by official methods.

In determining the adsorption of the various ions by hydrogel of silica, 200 cc. of each of the solutions of the salts was mixed with 50 gm. of the wet hydrogel of silica which had first been washed as free as possible of sulfates and which

contained 43.7 per cent moisture. These mixtures were placed in 250-cc. ground-glass stoppered bottles and shaken for 72 hours. At the end of this time it was found that equilibrium had been reached. The mixtures were then allowed to settle until clear or, if a hydrosol was produced which would not settle, a Pasteur filter was used. Aliquots were then pipetted from the clear, supernatant liquid or filtrate and analyzed by the same methods as before the hydrogel had been mixed. The pH value of each of the solutions was also determined.

In the case of the hydrogel of iron, the same procedure was followed with the exception that 25 gm. of the wet hydrogel containing 95.5 per cent moisture, was used.

EXPERIMENTAL RESULTS

In the following tables, adsorption is indicated in milligrams adsorbed per gram of dry hydrogel. The negative sign denotes negative adsorption. All others are positive.

TABLE 2
Adsorption of potassium by the hydrogel of silica

NUMBER OF SOLUTION	KNO ₃			K ₂ SO ₄		
	Reaction	K ADSORBED PER GRAM GEL		Reaction	K ADSORBED PER GRAM GEL	
		Dry	Wet		Dry	Wet
	pH	mgm.	mgm.	pH	mgm.	mgm.
1	9.873	11.2	11.45	9.890	10.06	10.38
2	8.554	9.86	10.22	8.706	9.93	10.19
3	6.830	2.60	3.68	7.337	4.70	5.37
4	6.390	-1.07	0.37	7.117	1.91	2.79
5	3.360	-1.332	0.114	5.275	1.16	2.36

Results shown in the "dry" column were calculated not on a volume of 200 cc. of the solution, but on 200 cc. plus the amount of moisture contained in the hydrogel at the time it was added. Results shown in the "wet" column were calculated on a basis of 200 cc., the amount of the salt solution added. It will be noted that the amount of the positive potassium ion adsorbed decreased with a decrease of pH or OH concentration, showing the greatest adsorption took place in an alkaline solution, while the negative phosphate ion was adsorbed to a greater extent at high hydrogen-ion concentrations. This may be seen more plainly by referring to the curves on figure 1 of the authors' previous paper (4).

The adsorption of the potassium varied with the hydrogen-ion concentration as in table 1 where potassium acid phosphate was used. The curves in the above mentioned figure show how similar the adsorption of the potassium with the three salts was. Near the neutral point a small change in the pH value made a marked change in the amount of potassium adsorbed.

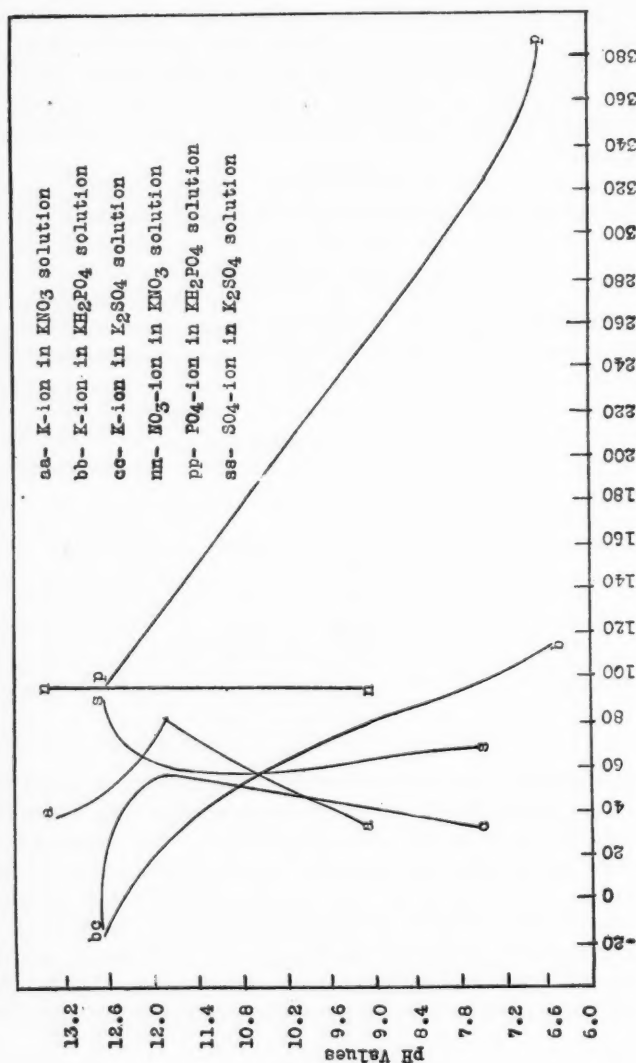


FIG. 1. ADSORPTION OF K, NO_3 , PO_4 , SO_4

Table 3 gives the adsorption by hydrogel of silica in solutions of $\text{CaH}_4(\text{PO}_4)_2$.

In the case of calcium salts it was impossible to obtain a wide range of pH values due to the precipitation of $\text{Ca}(\text{OH})_2$ by NaOH . In table 3 it will be noted that solutions 2 and 3 had the same pH value. Solution 2 contained a small amount of NaOH and HCl in equal quantities, while solution 3 contained only the salt $\text{CaH}_4(\text{PO}_4)_2$.

The calcium ion was adsorbed in the same order as the potassium ion in the potassium salts, but the order of adsorption of the phosphate ion in this case was reversed over that in the potassium phosphate.

TABLE 3
Adsorption of $\text{CaH}_4(\text{PO}_4)_2$ by the hydrogel of silica

NUMBER OF SOLUTION	REACTION	Ca ADSORBED PER GRAM GEL		PO ₄ ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	mgm.	mgm.	mgm.	mgm.
1	4.243	0.45	1.09	-0.64	2.56
2	4.193	0.41	1.05	-0.75	2.47
3	4.193	0.36	1.01	-1.00	1.87
4	4.142	0.18	0.85	-1.56	1.72
5	2.463	-0.89	-0.12	-2.62	0.77

TABLE 4
Adsorption of $\text{Ca}(\text{NO}_3)_2$ by the hydrogel of silica

NUMBER OF SOLUTION	REACTION	Ca ADSORBED PER GRAM GEL		NO ₃ ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	mgm.	mgm.	mgm.	mgm.
1	6.695	0.61	1.25	-3.32	-0.88
2	6.120	-0.86	-0.07	-3.32	-0.88
3	6.086	-1.49	-0.64	-0.86	-1.33
4	6.052	-1.57	-0.71	-1.35	0.88
5	2.284	-2.86	-1.52	-1.35	0.88

The adsorption of calcium followed the same order as potassium, but the adsorption of the nitrate ion did not seem to be influenced to any great extent by the hydrogen-ion concentration though the general tendency was to decrease with a decrease in hydrogen-ion concentration. The maximum adsorption occurred in the solution which contained neither NaOH nor HCl . Solutions 1 and 2 contain .002 *N* and .0002 *N* NaOH , solution 3 contained only the salt, and solutions 4 and 5 contain .002 *N* and .0002 *N* HCl . It would appear that the presence of these compounds influenced the adsorption of the nitrate ion.

Tables 5, 6, 7, 8, and 9 give the adsorption of salts by the hydrogel of ferric hydroxide. The pH values in the case of this gel were made much higher than

the corresponding pH values for the silica gel, in order to avoid a chemical solution with the hydrogel.

Table 5 shows that the negative phosphate ion is adsorbed to the greatest extent in the lower hydroxyl-ion concentration and decreases as the hydroxyl-ion concentration increases. But the positive potassium-ion does not follow the general rule of the positive ion. This is the only case found so far where the positive ion adsorption increases with the decrease of pH values throughout.

TABLE 5
Adsorption of KH_2PO_4 by the hydrogel of ferric hydroxide

NUMBER OF SOLUTION	REACTION	K ADSORBED PER GRAM GEL		PO ₄ ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	mgm.	mgm.	mgm.	mgm.
1	12.730	-65.2	-16.3	9.3	97.2
2	11.868	-14.3	29.2	51.2	135.0
3	7.439	63.0	99.0	216.0	324.1
4	6.695	77.7	111.2	320.9	378.1
5	6.627	80.9	114.1	329.9	386.2

TABLE 6
Adsorption of KNO_3 by the hydrogel of ferric hydroxide

NUMBER OF SOLUTION	REACTION	K ADSORBED PER GRAM GEL		NO ₃ ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	mgm.	mgm.	mgm.	mgm.
1	13.389	-0.5	39.9	34.4	97.8
2	12.612	11.0	50.2	34.4	97.8
3	11.868	48.9	84.0	34.4	97.8
4	9.417	0.2	40.4	34.4	97.8
5	9.281	-4.9	36.2	34.4	97.8

From table 6 it is seen that the adsorption of the anion was not affected by a change of pH values but that its adsorption rate was independent of other ions present. The cation seemed to present a maximum adsorption in this case around a pH value of 11.868. The same will be noticed in table 7 which shows a minimum adsorption of the sulfate ion at the same pH value. Curves in figure 1 make this more plain.

The maximum adsorption of the potassium ion at a pH value of 11.530, and the minimum adsorption of the sulfate ion at the same hydrogen-ion concentration is worthy of note. This is better shown by the curves in figure 1.

In table 8 we find the greatest adsorption throughout that has been noticed so far. While the change of pH values seems to have had but little effect on the adsorption of the phosphate ion, it has a tendency to present a minimum

adsorption as was noted in table 7. The cation here followed the general order of cation adsorption with a change of pH value.

It will be seen in table 9 that there was slightly greater adsorption in solution 5 than in solution 4, which breaks the order of the cation adsorption. This is accounted for by the fact that solution 5 suffered such a high peptiza-

TABLE 7
Adsorption of K_2SO_4 by the hydrogel of ferric hydroxide

NUMBER OF SOLUTION	REACTION	K ADSORBED PER GRAM GEL		SO ₄ ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	mgm.	mgm.	mgm.	mgm.
1	12.645	-60.8	-14.5	44.2	91.1
2	12.578	-01.0	38.9	21.2	70.5
3	11.530	21.7	59.2	10.1	60.7
4	8.182	-02.4	37.7	19.1	68.9
5	7.624	-07.8	32.8	19.1	68.9

TABLE 8
Adsorption of $CaH_4(PO_4)_2$ by the hydrogel of ferric hydroxide

NUMBER OF SOLUTION	REACTION	Ca ADSORBED PER GRAM GEL		PO ₄ ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	mgm.	mgm.	mgm.	mgm.
1	5.156	109.5	118.6	554.8	589.0
2	5.156	104.5	114.1	536.8	572.8
3	5.038	101.9	111.8	536.8	572.8
4	5.021	101.9	111.8	581.7	613.3
5	4.446	56.4	71.2	587.7	618.7

TABLE 9
Adsorption of $Ca(NO_3)_2$ by the hydrogel of ferric hydroxide

NUMBER OF SOLUTION	REACTION	Ca ADSORBED PER GRAM GEL		NO ₃ ADSORBED PER GRAM GEL	
		Dry	Wet	Dry	Wet
	pH	mgm.	mgm.	mgm.	mgm.
1	6.036	29.3	47.6	-12.0	62.0
2	5.241	-03.6	18.3	-12.0	62.0
3	5.122	-39.1	-13.3	-12.0	62.0
4	5.038	-46.4	-20.0	-12.0	62.0
5	4.432	-31.5	-06.5	-12.0	62.0

tion, that the accurate determination of the adsorption became very difficult. Here again we notice the general tendency of the nitrate ion not to be affected by the pH value.

Inasmuch as it seems to be the general tendency for an acid reaction to fasten the negative ion and an alkali reaction to fasten the positive ion, we believed

that by reversing the charge on the gel we should get a release of the ions which were adsorbed the most strongly. That is to say, by washing a gel which had adsorbed a negative ion strongly with an alkali, the ion should be released or washed out. So we proceeded in the following manner.

The silica gels from solutions 1, 2, 4 and 5 of table 1 (Absorption of KH_2PO_4 by hydrogel of silica) were used. Solutions 1 and 2 contained or had absorbed a relatively large amount of the potassium while solutions 4 and 5 had absorbed the phosphate ion. All the samples were then placed in filter papers in separate funnels and washed with water to remove that portion of solution mechanically held. Analysis of these portions were made as shown in the results. After the analysis showed only a small quantity of potassium and phosphate ions in the wash water, the acid and alkali washings were begun. For this, 0.1*N* HCl or NaOH were added in 50-cc portions. The filtrates were collected in separate beakers. To gel 1, 1 *N* HCl was added; to gel 2, 0.1*N* NaOH; to gel 4, 0.1*N* HCl and to gel 5, 1.0*N* NaOH. Three successive samples of washings were collected from each of the gels, then a fourth portion was added to each and allowed to stand over night. This was run off the next morning and analyzed along with the others. Table 10 gives the results obtained from the analyses of the HCl and corresponding previous water washings.

TABLE 10
Analysis of HCl washings of hydrogel of silica

WASHING	GEL NO. 1 K RELEASED	GEL NO. 4 PO_4 RELEASED
	mgm.	mgm.
1st 50 cc. H_2O	15.52	85.31
3rd 50 cc. H_2O	0.83	7.33
1st 50 cc. HCl.....	22.91	5.19
2nd 50 cc. HCl.....	21.96	2.70
3rd 50 cc. HCl.....	19.84	1.63
Over night HCl.....	24.37	8.69

TABLE 11
Analysis of NaOH washings of hydrogel of silica

WASHING	GEL NO. 2 K RELEASED	GEL NO. 5 PO_4 RELEASED
	mgm.	mgm.
1st 50 cc. H_2O	20.67	95.65
3rd 50 cc. H_2O	1.66	1.09
1st 50 cc. NaOH.....	2.94	2.70
2nd 50 cc. NaOH.....	3.65	1.35
3rd 50 cc. NaOH.....	2.59	1.09
Over night NaOH.....	14.77	7.88

Table 11 gives the results obtained from the analyses of the NaOH and corresponding water washings.

It will be seen from table 10 that the HCl washing caused a marked release of the potassium ion, whereas no increase was noticed in the phosphate ion until the HCl was left overnight in contact with the gel.

In table 11 it will be noticed that the NaOH caused a slight increase in the liberation or movement of the phosphate ion. The small increase was probably due to the small amount present to begin with. We also obtained a

slightly increased movement of the potassium ion. This is accounted for by a replacement of the potassium by the sodium ion until a state of equilibrium is reached. The explanation becomes even more plausible when the results for the overnight washings are observed.

DISCUSSION

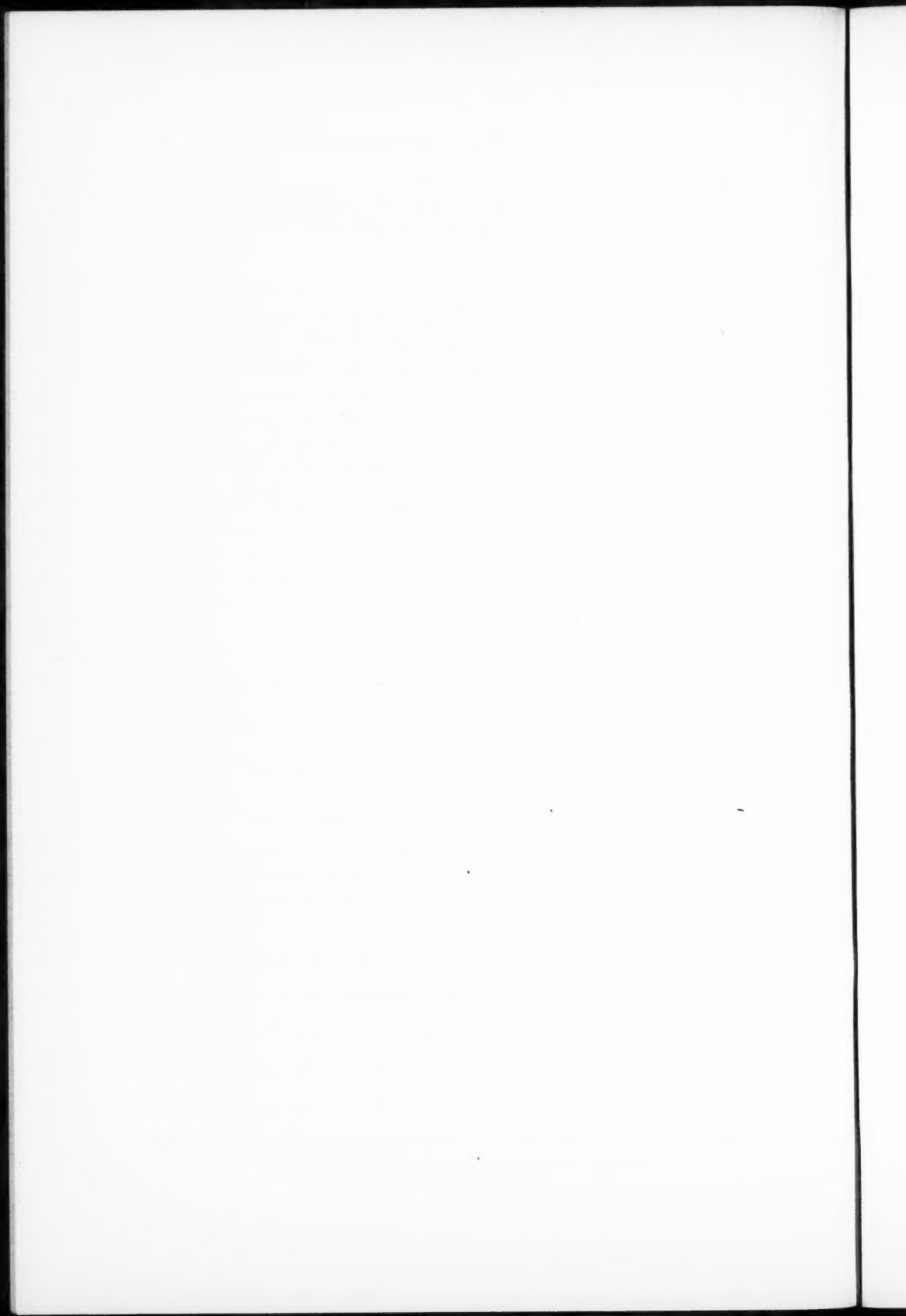
We had expected when starting the work that an increase in the concentration of the hydroxyl ions would increase the adsorption of cations by soil colloids. These results were very generally found as seen in the preceding tables. This was anticipated in accordance with Helmholtz's electrical double-layer theory which assumes that one ion may become more closely associated with the colloidal particle than the other ion. This most closely associated ion would impart its charge to the colloidal particle, but this charged colloid and ion will attract ions of opposite charge and in our case, if it is the OH ion that is giving the charge to the colloid, it should attract the metal ions which have the opposite sign. Hence, the solution should become poorer in metallic ions. This was what was found, but this is only one explanation of the general behavior of the metallic ions. The negative ions showed many specific cases, and work is now in progress to explain the entire phenomenon in terms of chemical reactions which are governed by difference of solubility or hydrogen-ion concentration.

SUMMARY

1. The adsorption of the cation as a rule increased with increased hydroxyl ion concentration, or with increased pH value.
2. The adsorption of the phosphate ion increased with decreased pH value in some cases.
3. Adsorption of the nitrate and sulfate ions was not consistently influenced by the reaction of the solutions.
4. Anions seem to be adsorbed in the order of phosphate, sulfate and nitrate with phosphate well in the lead.
5. Adsorption by ferric hydroxide gel is greater than by silicic acid gel.
6. There is a large change in adsorption of potassium ions for very slight change in the hydrogen-ion concentration around the neutral point.

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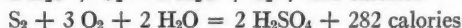
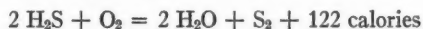
OXIDATION OF ZINC SULFIDE BY MICROÖRGANISMS¹

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Under conditions of natural exposure, hydrogen sulfide and metallic sulfides are slowly oxidized. In a fine state of subdivision, and in the presence of catalytic agents oxidation proceeds and sulfuric acid is formed. The sulfuric acid in turn reacts with the bases to form sulfates. Winogradsky (10, 11) described a group of sulfur bacteria which were able to reduce H_2S to elementary sulfur, and to oxidize elementary sulfur to H_2SO_4 . The sulfur transformation is given in the following reactions:



While sulfur oxidation in soils appears to be chiefly a biological phenomenon, there seems no doubt that the results are influenced by purely chemical reactions.

Kappen and Quensell (4), Van Bemmelen (9) and MacIntire and his associates (6) conclude that sulfur oxidation takes place without the aid of bacteria. Boulanger (1), Demolon (2), Lipman and his assistants (5) have laid much emphasis on the biological factor in sulfur oxidation in the soil. Lipman seized upon the idea of utilizing this factor for agricultural purposes. Rock phosphate was mixed with flowers of sulfur, and the mixture inoculated with sulfur-oxidizing organisms. The sulfur, transformed into H_2SO_4 , reacts with the $\text{Ca}_3(\text{PO}_4)_2$ rendering the inert phosphates soluble and available to plants.

Little is known about the influence of microorganisms upon the oxidation of metallic sulfides. Rudolfs (7) found that certain sulfur-oxidizing bacteria readily oxidize pyrites. As far as the writers were able to learn, nothing else is on record as to possible biological reactions involving metallic sulfides.

PROCEDURE

With a mixed culture of a large group of sulfur-oxidizing organisms we finally succeeded in securing a culture which was able to slowly attack precipitated ZnS . From these cultures the best was selected, inoculated into a sterilized soil medium, and mixed with a nearly pure Spanish zinc blende. This culture served as inoculum in mixtures of unsterilized soil with two commercial zinc sulfides. The cultures were made by thoroughly mixing air-dry portions of soil, blende, and in some cases flowers of sulfur. The soil used was Penn loam. Inocula-

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tions were made with a water extract of the original culture in portions of the new material. The mixtures consisted of:

NUMBER	SOIL	ORE	SULFUR	INOCULATION
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	
1	250	75	None	None
2	250	75	None	Soil
3	250	75	10	Sulfur
4	245	75	10	Blende
5	240	75	25	Soil

Triplicate composts were placed in tumblers covered with glass plates and incubated at 28°C. Each culture was weighed and the water-holding capacity determined. Samples were weighed out and relative acidities, hydrogen-ion concentrations, sulfates and soluble zinc determined. The composts were kept at approximately 60 per cent of the water-holding capacity throughout the period of incubation. The amount of water lost by evaporation determined by placing the tumblers on the scale pan, was added each week and the mixtures removed from the tumblers to provide for sufficient aeration.

Water-extractions were made of weighed air-dry samples from each culture, by shaking 20 gm. of mixture with 200 cc. of diluted water in 1-liter flasks in a shaking machine for 2 hours. The flasks were left standing over night and an aliquot drawn off for the pH determinations. The remainder of the contents of the flasks were then filtered until the liquid was clear.

The hydrogen-ion concentrations were determined by the colorimetric method, with the apparatus described by Van Alstine (8).

TABLE 1
Analyses of zinc ores

ORE	Zn	Fe	CaO	S	Pb	SO ₄	RE-ACTION	MOIST-URE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>pH</i>	<i>per cent</i>
Zinc concentrates.....	46.6	3.9	4.9	29.2	0.12	0.037	7.4	0.14
Zinc blende.....	48.4	7.7	2.7	31.2	0.20	0.060	7.7	0.64
Penn loam.....		9.4	0.32	0.04		0.119	6.7	2.34

Determinations of relative acidity were made upon aliquots of the water extract. The liquid was boiled to expell CO₂, cooled, and titrated with 0.1 *N* NaOH, using phenolphthalein as indicator.

The sulfates were determined by acidifying aliquots of the water extract with concentrated HCl and precipitating at the boiling point with BaCl₂.

Soluble zinc was determined by the electrolytic method proposed by Classen (3) and by the volumetric method with ferrocyanide solution, using uranium acetate as indicator. Iron, etc., was removed before titration. The results recorded are calculated to the moisture-free basis of the soil and ores. The analyses of the ores and soil at the beginning of the investigation are given in table 1.

EXPERIMENTAL RESULTS

For a study of the progress of the reaction, details of two series of cultures are given in tables 2 and 3. The water-soluble acidity increased continuously,

while the hydrogen-ion concentration fluctuated. In general the hydrogen-ion concentration shows a sudden increase, then a slow decrease, and again an increase. Determinations of pH values do not show the total acidity in the

TABLE 2
Reaction of mixtures after various periods of incubation

CULTURE NUMBER	INITIAL		1 WEEK		2 WEEKS		3 WEEKS		6 WEEKS		9 WEEKS		12 WEEKS		18 WEEKS	
	cc.*	pH	pH	pH	cc.	pH	cc.	pH	cc.	pH	cc.	pH	cc.	pH	cc.	pH
1	0.0	7.2	7.1	7.1	0.1	7.2	0.1	7.0	0.1	7.1	0.4	6.6	0.6	6.4		
2	0.0	7.2	7.0	6.9	0.3	6.6	0.6	6.4	1.0	6.2	2.6	5.2	5.2	5.0		
3	0.0	7.2	6.6	3.5	12.0	3.2	21.2	3.7	22.8	4.0	27.6	3.4	55.6	3.5		
4	0.1	6.9	6.5	4.2	4.6	3.2	18.4	3.4	19.6	4.0	25.4	3.3	50.8	3.7		
5	0.0	7.1	6.1	3.7	10.8	3.1	24.8	3.6	27.2	3.1	52.4	2.9	79.6	3.0		
6	0.0	7.2	7.1	7.0	0.0	7.1	0.0	6.7	0.1	6.6	0.2	6.7	0.3	6.5		
7	0.0	7.2	7.1	6.6	0.4	6.6	2.0	5.7	3.0	4.6	10.6	4.7	30.2	4.6		
8	0.0	7.1	6.8	6.7	0.6	6.5	8.0	4.5	19.2	4.6	32.0	4.4	63.2	4.4		
9	0.0	7.0	6.7	6.6	0.4	6.7	7.4	4.8	17.2	4.6	34.0	3.8	72.4	4.1		
10	0.0	7.1	6.3	6.7	1.0	6.4	14.6	3.8	26.0	4.5	42.0	4.3	62.8	4.0		

* Acidity expressed in cc. 0.1 *N* NaOH required to neutralize acidity of water extract from 10 gms. of mixture.

TABLE 3
Accumulation of water-soluble zinc and sulfate
Per 10 grams of mixture

CULTURE NUMBER	INITIAL		AFTER 1 WEEK		AFTER 3 WEEKS		AFTER 6 WEEKS		AFTER 9 WEEKS		AFTER 12 WEEKS		AFTER 18 WEEKS		PROPORTION OF TOTAL ZINC RECOVERED SOLUBLE
	SO ₄	Zn	SO ₄	Zn	SO ₄	Zn	SO ₄	Zn	SO ₄	Zn	SO ₄	Zn	SO ₄	Zn	
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	
1	5.0	3.2	5.4	2.9	7.0	3.1	13.8	7.9	29.0	12.6	33.4	16.4	50.6	18.0	1.67
2	5.1	3.7	13.4	6.4	24.2	12.2	58.2	34.7	102.1	51.4	114.2	63.0	142.2	98.0	8.32
3	10.1	3.6	17.2	12.8	146.4	90.6	198.9	134.5	256.2	157.4	282.0	172.6	377.8	190.0	19.17
4	11.1	3.8	18.1	19.2	133.1	77.7	182.8	149.4	186.4	150.6	228.8	180.0	364.2	192.5	17.59
5	18.8	3.7	35.6	18.2	121.8	64.4	202.1	191.1	220.1	204.0	358.3	297.2	518.1	369.0	37.56
6	5.4	3.1	5.4	3.3	7.6	4.1	21.9	15.8	43.8	30.4	81.8	30.6	139.2	67.5	5.81
7	5.4	3.2	6.8	5.6	37.9	6.2	53.0	17.6	62.9	40.7	141.3	81.0	271.8	193.3	17.65
8	19.1	3.0	41.9	6.2	151.5	102.9	195.1	159.8	258.7	166.2	327.0	195.2	488.0	255.0	23.82
9	14.2	3.1	19.3	6.2	138.4	89.1	196.8	101.5	253.4	123.3	342.0	194.4	545.8	270.0	25.42
10	18.7	3.1	27.9	6.1	137.4	93.1	211.6	159.8	301.8	191.5	393.1	198.2	505.0	245.0	23.76

mixtures, but merely the strength of the free acid at the time the determinations were made. These determinations serve as a convenient means of following the process of oxidation and the general reaction. The sudden increase marks a rapid oxidation of sulfur to free H₂SO₄, which in turn reacts with the

basic constituents of the mixtures. The decrease of hydrogen-ion concentration indicates a more complete reaction. As may be seen from table 3, sulfur oxidation takes place in all cultures.

Apparently as the result of chemical reactions a small amount of zinc sulfide was converted into zinc sulfate. This agrees with the findings in sterilized cultures which had been incubated for 30 weeks. However, the inoculated cultures made far more zinc soluble than the uninoculated mixtures; while mixtures to which elementary sulfur was added showed still greater amounts of soluble zinc. This seemed to indicate that in mixtures containing high proportions of soluble zinc, sufficient sulfuric acid was produced by the sulfur-oxidizing organisms to react with the zinc ore.

TABLE 4
Soluble zinc from ores incubated for a period of 18 weeks

TREATMENT OF MIXTURE OF 250 GM. SOIL AND 75 GM. ORE	WILLEMITE	ZINC CARBONATE	LOW GRADE SILICATE ORE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Uninoculated.....	0.41	1.02	2.10
5 gm. inoculated soil.....	0.23	1.14	2.06
10 gm. inoculated sulfur.....	15.53	16.03	74.91
5 gm. inoculated blende and 10 gm. sulfur.....	6.81	19.73	47.27
10 gm. inoculated soil and 25 gm. sulfur.....	9.99	22.67	68.44

A number of experiments were then made with other zinc ores, notably with Willemite (Zn_2SiO_4), Smithsonite (ZnCO_3) and a low silicate ore. The cultures were inoculated with a pure strain of sulfur-oxidizing organisms, known to convert sulfur into sulfuric acid and analyzed as described above.

The results at the end of 18 weeks are given in table 4.

It is evident from table 4 that the sulfuric acid produced by the oxidation of elementary sulfur readily transformed these different ores.

It is a point of interest to know at which hydrogen-ion concentration transformation of ZnS to ZnSO_4 takes place. To find the exact acidity necessary to change the different zinc ores, curves were constructed from readings of pH values obtained by additions of different amounts of 0.1 N H_2SO_4 to the ores. Ten grams of ore were shaken for 2 hours in a shaking machine with definite amounts of sulfuric acid added to distilled water to make 200 cc. of liquid. The bottles with contents were left standing for 24 hours and an aliquot of the supernatant liquid was then drawn off and the hydrogen-ion determinations made. At the critical points sufficient determinations were made to check up all points obtained. The results for one zinc sulfide ore are graphically shown in figure 1. It is apparent that at approximately pH 5.7 to 5.5 there occurred a sudden increase in the solubility of the ZnS , indicating that but very weak acidity is required to reach to critical point of solubility. However, with even weaker acidity a rapid reaction takes place. If this graph is compared with the figures in the tables, the relation between the hydrogen-ion concentration and ZnSO_4 formation is strikingly shown.

Since a possible practical application would necessarily be directed toward the low grade ores, the part of the work dealing with the low grade silicate ore was most interesting. However, an addition of elementary sulfur to such ores would considerably increase the cost of operation, and no profitable use could be made of this biological method.

Studies now under way may result in reducing the incubation period, which in the above tests had to be prolonged.

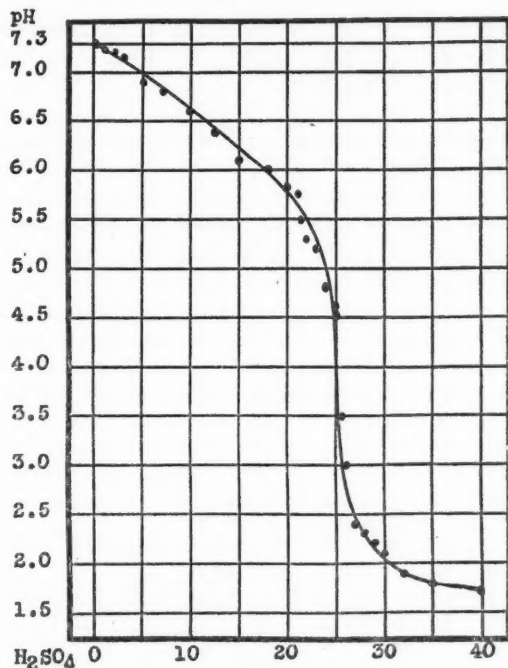


FIG. 1. GRAPH SHOWING AT WHICH HYDROGEN-ION CONCENTRATION THE ZINC OF ZINC SULFIDE BECOMES SOLUBLE

From a practical view point it seems more interesting to develop a strong oxidizing-culture which needs little or no free sulfur with which to start. This would limit the work to low-grade sulfides. Since enormous quantities of low-grade sulfide ores are found and are at present rarely used, this point was therefore investigated.

Low grade zinc ores are frequently associated with other sulfides, so much so, in fact, that economic geologists usually consider lead sulfide and zinc sulfide together.

A study was made with rather low-grade zinc sulfide containing equal amounts of zinc sulfide and of galena (PbS). Mixtures were made of 50 parts of

soil, 50 parts of zinc sulfide (14.5 per cent Zn), and 50 parts of galena. Sulfur was added to some mixtures as to the cultures reported in table 1; uninoculated check cultures and mixtures with zinc sulfide and galena alone were included in the series. After 12 weeks, 24.5 per cent of the zinc was soluble in the cultures to which sulfur had been added, while 10.1 per cent zinc was soluble in the inoculated mixtures without sulfur. No trace of lead was present in any of the extracts. After 30 weeks, 72.4 per cent zinc was soluble in mixtures without sulfur and a trace of lead appeared.

From the data presented the following tentative conclusions may be drawn:

1. Microorganisms are able to transform zinc sulfide to zinc sulfate.
2. The growth of these organisms is not inhibited by the resulting soluble zinc.
3. The addition of elementary sulfur to impure cultures increases the rate of solubility of zinc blende.
4. The "Lipman" sulfur-oxidizing organisms produce sufficient H_2SO_4 from elementary sulfur to render zinc carbonate and zinc silicate soluble.
5. A possible biological method can be worked out for economical utilization of low-grade zinc sulfide ores.

Studies are under way aimed to reduce the amount of soil or to supplant it by a suitable medium; to determine the influence of temperature and moisture-content of the mixtures; and to purify the bacteria which use the combined sulfur as a source of energy. These studies will be reported on subsequently.

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MODIFICATIONS OF THE SOIL FLORA INDUCED BY APPLICATIONS OF CRUDE PETROLEUM¹

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Although crude petroleum is not normally used as an antiseptic or germicide, it undoubtedly has a certain amount of germicidal action and its action in the soil is in all probability very largely that of an antiseptic.

In addition to this action, several investigators have shown that certain bacteria have the power of utilizing the hydrocarbons contained in crude petroleum as sources of carbon and energy. Crude petroleum therefore may modify the soil flora in much the same way as does large applications of carbohydrates, such as straw.

The literature contains very few references to the effect of crude petroleum on the soil flora and biochemical reactions of the soil. However a great deal of work has been carried out in studying the action of various other materials used as soil antiseptics. The materials most commonly used in this connection have been carbon disulfide, ether, toluene and benzene. While the data, with respect to the action of these substances, are largely conflicting, the majority of investigators have reported a temporary decrease in bacterial numbers and biochemical activities, later followed by a large increase.

Rahn (4) reported in 1906, the presence in soil of a mold belonging to the *Penicillium* group which was capable of breaking down paraffin and using it as a source of food.

Sohngen (5), a number of years later, gave an extensive report of a number of bacteria isolated from soil which are capable of using paraffin, petroleum and benzene as sources of carbon and energy.

Tausz and Peter (6), in an extensive report on methods of analysis of hydrocarbons, describe several organisms capable of utilizing definite hydrocarbons as sources of carbon and energy.

Gainey (2), in an article discussing the effect of paraffin on ammonification and nitrification in the soil, concludes that these biochemical activities are very materially hindered by the presence of this substance.

Carr (3), in making applications of crude petroleum to soil growing soy beans, found that their growth was apparently improved through the addition of small amounts of crude petroleum (up to 0.75 per cent), and that rather large amounts could be mixed with the soil (4.0 per cent) before it killed the soy bean plant.

¹ Part of a thesis submitted to the faculty of Purdue University in partial fulfillment of the requirements for the degree of Master of Science in Agriculture.

This opportunity is taken to express to Dr. R. H. Carr, due appreciation for his suggestions and criticisms of this work.

TECHNIC

Crude petroleum having a specific gravity of 0.8370 was used in all of the investigations made. The sample was secured from the Indiana Pipe Line Company at the Kankakee Pumping Station, just as it came from the pipe line, and was kept in a closed vessel at all times in order to avoid any outside contamination.

The methods of making the application of this crude petroleum to the soil varied with the type of experiment being conducted and will be described in detail in connection with the various experiments.

Bacterial counts of the soil samples were made by plating on agar. A number of different media recommended by various investigators were tried out in an effort to find the one which would prove the most satisfactory both from the standpoint of the number of colonies developing and the production of characteristic colonies. Although the media did not vary considerably in their degrees of suitability, it was finally decided that a synthetic medium of the following composition and adjusted to a hydrogen concentration of pH 7 was the most satisfactory.

Sodium potassium tartrate.....	5.0 gm.
Peptone.....	1.0 gm.
Dibasic potassium phosphate.....	0.5 gm.
Magnesium sulfate.....	0.2 gm.
Agar.....	15.0 gm.
Distilled water.....	1 liter

Ammonia was determined by direct distillation with magnesium oxide. It is recognized that this method of determination may give results which are slightly high, because of the breaking down of certain amides and amino acids into ammonia. The results are, however, comparable one with another if the same technic is used in all cases.

Nitrate determinations were made by the phenol disulfonic acid method, as modified by Noyes (3) for soil analysis. When the work was first started and the complete absence of nitrates was noted in the soil treated with the crude petroleum, it was thought that the presence of the crude petroleum might interfere with the colorimetric determination of nitrates. To check this point, a known quantity of nitrate was added to a sample of soil containing a heavy application of the crude petroleum, and the nitrate was recovered quantitatively by the phenol disulfonic acid method. In addition, samples showing no nitrates by the phenol disulfonic acid method were also analysed for nitrates by the Devardo method and the same results were obtained. It is felt that the method used is very well adapted to the work, since extremely small amounts of nitrate may be detected.

Crude petroleum was determined in the soil by extraction with equal parts of petroleum and ethyl ethers. The material extracted was dried at a temperature of 37.5°C. for 24 hours and then placed over calcium chloride to complete the drying process. The drying was carried out in this manner to avoid any unnecessary loss of the more volatile portions from the crude petroleum. Blanks on untreated soil were determined in order to make correction for the material other than crude petroleum which might be extracted.

FIELD PLOT EXPERIMENTS

For this part of the work a healthy normal row was selected from an experimental corn plot. This corn received no treatment other than the application of crude petroleum. The soil was a dark silt loam containing about 7 per cent volatile matter, and the corn was of the yellow dent variety. The crude petroleum was applied in varying amounts to the individual hills of corn, after it was about 1 foot high. This application was made to the surface of

the soil in a circle about the hill at a distance of about 6 inches from the plants. The row and the hills selected for this experiment were as representative and normal as it was possible to get them. Applications ranging from 25 cc. to 425 cc. were made to each hill.

Samples were taken from each of the treated hills at two different dates and moisture, nitrates, nitrification and bacterial counts determined.

Both aerobic and anaerobic bacterial counts were secured. The anaerobic count was secured by incubating the plates in an atmosphere of flowing hydrogen.

Nitrification was determined by setting 100 gm. of the soil away in a tumbler for a two weeks' incubation period. Nothing other than water to bring the moisture content to optimum was added to the soil.

TABLE 1
Field plot experiment

CRUDE PETROLEUM PER HILL	AUGUST 7, 1920				OCTOBER 16, 1920			
	Nitrates, on dry basis		Bacteria per gram of dry soil		Nitrates, on dry basis		Bacteria per gram of dry soil	
	Fresh samples	After 2 weeks incubation	Aerobes	Anaerobes	Fresh samples	After 2 weeks incubation	Aerobes	Anaerobes
	p.p.m.	p.p.m.	millions	millions	p.p.m.	p.p.m.	millions	millions
0	22.41	56.30	1.67	0.178	26.74	73.20	2.81	0.184
25	2.52	16.80	2.52	0.173	13.42	19.92	2.84	0.192
75	1.17	5.50	3.25	0.135	6.78	16.81	3.02	0.178
125	0.33	3.37	3.38	0.176	4.78	14.35	3.35	0.164
175	0.00	1.67	2.88	0.209	1.79	3.42	3.21	0.192
225	0.00	1.67	3.28	0.201	0.00	1.68	3.78	0.135
275	0.00	1.68	3.92	0.209	0.00	1.68	3.91	0.186
325	0.00	1.67	4.69	0.194	0.00	1.67	4.73	0.203
375	0.00	1.25	4.78	0.197	0.00	1.25	4.53	0.211
425	0.00	0.75	5.24	0.203	0.00	1.25	6.78	0.203

By examination of table 1, it will be seen that the nitrate content of the fresh soil was materially lowered in both sets of samples. Also the quantity of nitrate produced during two weeks' incubation was lessened in proportion to the size of the application of crude petroleum. In comparing the two sets of samples, it is seen that the soil had in a measure regained its power of nitrate production at the time the last set of samples was taken.

Total counts of aerobic bacteria were much increased by the applications of crude petroleum, the increase being greater with the larger applications of crude petroleum. Although total aerobic counts were slightly higher at the second date of sampling, the percentage of increase, due to the application of crude petroleum, was no larger. Although there was an increase in the total aerobic count of bacteria, the number of bacterial types was very greatly reduced by the higher applications of crude petroleum.

Applications of crude petroleum seemed to have little effect on the number of organisms developing under anaerobic conditions.

Yield records on a plot of such small size are of very little value. However, it was noted that the largest and best ears of corn were produced on the hills which were treated with the highest amounts of crude petroleum. While the number of hills in each treatment is not large enough to give accurate crop data, this does indicate that the crude petroleum as applied in this experiment was not detrimental to plant growth and development.

LABORATORY STUDIES ON AMMONIFICATION AND NITRIFICATION

This experiment was designed to determine the effect of applications of crude petroleum on the biochemical activities of the soil flora as measured by ammonification and nitrification. Also an attempt was made to determine what modifications were brought about in the types of bacteria developing on agar plates.

A brown sandy loam from an old soy bean field in the Purdue Experimental Plots was used in this test.

Nine 100-gm. portions were placed in glass tumblers and 1 per cent of cotton seed meal added. To three of these portions 2 cc. of crude petroleum was added and to three others, 5 cc. was added. The crude petroleum in each case was thoroughly mixed with the soil. The tumblers were then set away to incubate for varying periods of time.

The initial sample and the incubated portions were all analyzed for nitrates, ammonia and bacterial counts.

Bacterial plates were incubated for 10 days and then counted and photographed in order to record the number of colonies and the uniformity of type.

The treatments of crude petroleum used in this experiment were intentionally made very high in an effort to intensify any effects which might be produced.

A study of table 2 shows the ammonia content of the soil to have remained about constant during the first 7 days of incubation, with a slight loss in the case of the treated samples. At the end of 17 days the ammonia content had risen very sharply and this increase continued over the succeeding 10 days of incubation.

Mold growth in all of these samples was very intense and the production of ammonia is probably, in part at least, due to this factor.

However, the untreated sample shows a greater ammonification at all stages than either of the others, indicating that the applications of crude petroleum slightly decreased the ammonifying efficiency of the soil.

Nitrate production was affected to a far greater extent than ammonia production. The formation of nitrates in the samples stopped and the initial nitrates of the soil disappeared.

In contrast to the lowered efficiency of ammonia production and the complete loss of nitrates, bacterial numbers are shown to have increased more

rapidly in the treated samples than in the untreated one. A careful study of table 2 shows, however, that the initial effect of the higher application of crude petroleum was to reduce the bacterial count, but that this period of initial decrease was followed by a period of very rapid multiplication.

Of equal interest is an observation of the types of bacteria developing in the treated and untreated samples. Plate 1 shows photographs of petri plate cultures made from various samples at the end of 17 days' incubation. The sample receiving no crude petroleum shows the development of a large number of different types of bacteria, as would be expected in the normal soil flora. Treated samples, however, show in addition to the increased

TABLE 2

Effect of applications of crude petroleum on bacterial counts, ammonification and nitrification

PERIOD OF INCUBA- TION	AMMONIA ON BASIS OF DRY SOIL			NITRATES ON BASIS OF DRY SOIL			BACTERIA PER GRAM OF DRY SOIL		
	No oil added	2 cc. oil added	5 cc. oil added	No oil added	2 cc. oil added	5 cc. oil added	No oil added	2 cc. oil added	5 cc. oil added
days	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	p.p.m.	millions	millions	millions
0	16.8			4.8			5.7		
7	15.9	15.7	9.1	16.0	3.2	2.2	4.8	10.0	2.6
17	687.0	619.1	450.4	48.0	0.0	0.0	16.5	22.6	34.4
27	775.0	587.3	480.9	80.0	0.0	0.0	28.0	37.8	57.3

number of colonies, a very marked reduction in the number of bacterial types. With the larger application there is at the end of 17 days' incubation, practically only one type of bacteria developing on the plates. Apparently some property of the crude petroleum has stimulated this particular bacterial type and at the same time has shown an inhibitory action on the other types originally present in the soil.

Mold growth was very intense in all of these samples as indicated by the presence of white hyphae throughout the soil.

GREENHOUSE EXPERIMENTS

In order that the bacteriological and biochemical activities of the soil as influenced by applications of crude petroleum might be studied in connection with crop growth, a series of experiments was carried out in the greenhouse using 2-gallon pots.

Since previous work had shown the transformation of nitrogen from protein to nitrate to be the chief biochemical factor affected, a rich black loam, high in organic matter was used, to which was added a sufficient quantity of the other fertilizer elements to insure a plentiful supply.

Two series of 15 pots each were set up. The series were duplicates, except for the fact that one received 25 gm. of dried blood per pot in addition to the other substances. Each pot contained 4000 gm. of soil, 20 gm. of calcium carbonate, 10 gm. of potassium chloride, and 15 gm. of calcium acid phosphate.

Crude petroleum was added to each series in varying amounts and was thoroughly mixed with the slightly moist soil before it was placed in the pots. The smallest application of crude petroleum used was 10 cc. per pot and the size of the treatment increased by increments of 10 cc. up to 100 cc. Between 100 cc. and 200 cc. the treatments were increased by increments of 20 cc.

Moisture conditions were kept as nearly optimum as possible but it was found very difficult to get the higher members of the series to take enough water.

At the end of 21 days and again at the end of 60 days, samples were taken from the pots and analyzed for nitrates and bacterial counts.

Decomposition of the dried blood was very intense and during the earlier part of the experiment the surface of the soil was dotted with white mold colonies.

An examination of tables 3 and 4 shows that the nitrate content of the soil in both series was lowered very materially by the application of crude petroleum. In the pots containing the larger applications of crude petroleum, no trace of nitrate could be found. The detrimental effect of the crude petroleum was not so marked in the dried blood series as in the series containing no dried blood. The retardation of nitrification by the crude petroleum treatments had worn off to a large extent at the date of the second and last sampling, i.e., after a period of 60 days.

Bacterial growth was apparently benefited by the applications of crude petroleum. The bacterial count increased with the size of the crude petroleum application in both series.

Uniformity of bacterial type was very marked at the time of the first sampling, particularly in the pots receiving the higher applications of crude petroleum. This degree of uniformity was partially lost in the lower pots of both series at the date of last sampling.

After these samples had been taken, the pots were planted with seed from a disease free ear of yellow dent corn. In addition to the original pots, three others were started. Each of these received the same amount of phosphorous, potassium and lime as did the original pots. In addition, one of these received dried blood and another received the nitrate treatments. No crude petroleum was added to these three pots.

Since it had developed that the nitrate production was very seriously interfered with, alternate pots in both series were treated with a solution of sodium nitrate, once each week, during the period of growth. Each pot received 1/8 gm. of sodium nitrate in a weak solution at each application.

The moisture-content was held at as nearly optimum as possible, and it was found that the higher members of the series took water more readily than they had previously.

Qualitative bacterial samples taken at the time of harvesting, and 115 days after treatment with the crude petroleum, still showed a marked uniformity

TABLE 3
Samples taken from greenhouse pots 21 days after treatment

CRUDE PETROLEUM APPLIED PER POT	POTS CONTAINING DRIED BLOOD			POTS WITHOUT DRIED BLOOD		
	Moisture	Nitrates in dry soil	Bacteria per gram of dry soil	Moisture	Nitrates in dry soil	Bacteria per gram of dry soil
cc.	per cent	p.p.m.	millions	per cent	p.p.m.	millions
10	19.4	121.0	8.61	18.3	3.94	2.46
20	19.4	60.0	18.53	17.2	3.95	2.18
30	18.9	35.5	19.07	18.3	1.95	2.65
40	19.6	28.0	16.89	18.5	1.95	3.38
50	18.4	15.0	15.84	17.4	0.00	3.49
60	18.6	15.1	16.21	19.3	0.00	3.71
70	18.6	10.9	21.07	18.7	0.00	3.98
80	21.3	11.1	16.03	19.5	0.00	4.46
90	18.9	7.98	17.28	17.9	0.00	4.28
100	18.6	7.97	24.85	19.3	0.00	4.57
120	18.4	7.96	25.21	17.8	0.00	6.02
140	19.2	3.94	23.29	15.9	0.00	5.83
160	17.6	0.00	26.58	14.2	0.00	6.64
180	17.0	0.00	24.38	13.5	0.00	7.28
200	16.4	0.00	31.02	12.3	0.00	7.29

TABLE 4
Samples taken from greenhouse pots 56 days after treatment

CRUDE PETROLEUM APPLIED PER POT	POTS CONTAINING DRIED BLOOD			POTS WITHOUT DRIED BLOOD		
	Moisture	Nitrates in dry soil	Bacteria per gram of dry soil	Moisture	Nitrates in dry soil	Bacteria per gram of dry soil
cc.	per cent	p.p.m.	millions	per cent	p.p.m.	millions
0				19.3	21.6	1.87
0	20.1	77.0	12.20			
0				18.3	20.8	1.90
10	18.7	816.0	3.41	18.3	8.8	2.21
20	17.4	400.0	4.36	17.6	8.9	2.35
30	20.1	256.0	4.68	19.4	6.4	2.70
40	19.4	296.0	5.14	18.6	4.1	2.68
50	18.9	336.0	4.81	17.3	4.0	2.91
60	17.8	344.0	5.33	16.5	3.2	3.13
70	17.3	312.0	5.96	17.9	1.6	3.30
80	18.5	264.0	6.17	16.9	0.0	2.86
90	18.7	183.0	6.43	17.6	0.0	2.69
100	18.1	144.0	5.32	17.3	0.0	2.95
120	18.9	96.0	4.59	17.3	0.0	2.74
140	18.8	88.0	6.31	18.6	0.0	3.62
160	16.4	94.0	7.28	17.2	0.0	3.75
180	17.6	92.0	5.69	15.3	0.0	4.21
200	17.1	87.0	6.87	14.6	0.0	5.14

of bacterial type in the pots receiving 100 cc. or over of the crude petroleum and not dried blood. In the dried blood series the pots which received 180 cc. and 200 cc. of crude petroleum showed uniformity of bacterial type. All other pots showed as large a number of bacterial types as would be expected in the normal soil flora.

TABLE 5
Petroleum extracted by ether from soils 115 days after treatment

CRUDE PETROLEUM ADDED PER POT	WITH DRIED BLOOD			WITHOUT DRIED BLOOD		
	Extract from 10 gm. dry soil	Extract minus blank	Petroleum recovered	Extract from 10 gm. dry soil	Extract minus blank	Petroleum recovered
cc.	mgm.	mgm.	per cent	mgm.	mgm.	per cent
0	4.1			3.6		
10	18.2	14.1	67.4	13.7	10.1	48.3
80	101.6	97.5	52.5	103.4	99.8	53.8
200	191.6	187.5	45.6	163.4	159.8	38.9

TABLE 6
Growth (dry weight of tops) of dent corn after 56 days

CRUDE PETROLEUM APPLIED PER POT	POTS CONTAINING DRIED BLOOD		POTS WITHOUT DRIED BLOOD	
	No nitrate	Nitrate added	No nitrate	Nitrate added
cc.	gm.	gm.	gm.	gm.
0			27.10	
0				22.40
0	41.40			
10	32.70		6.10	
20		26.10		17.90
30	23.50		1.70	
40		27.50		5.60
50	15.60		1.40	
60		35.00		4.50
70	28.60		7.65	
80		27.40		2.90
90	21.60		1.30	
100		23.60		3.10
120	12.65		4.30	
140		15.50		8.60
160	16.20		0.40	
180		7.45		5.40
200	9.85		0.30	

Qualitative nitrate tests, made with Brucin, gave large quantities of nitrates in all pots except those showing considerable uniformity of bacterial type. It seems that nitrates are not formed in the soil to any considerable extent, as long as the effect of the crude petroleum is strong enough to maintain the uniformity of bacterial type.

The extraction of the soil with ether, as shown in table 5, seems to indicate that approximately 50 per cent of the crude petroleum had either been volatilized or broken down into simple substances by the action of certain of the microorganisms found in the soil. Sufficient data have not been secured, however, to substantiate this point.

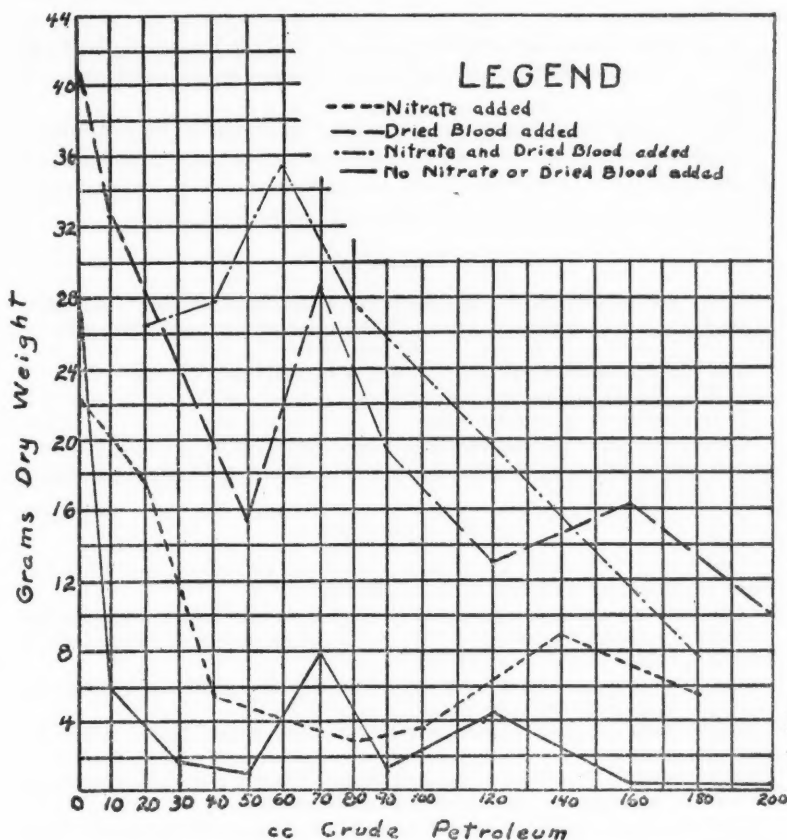


FIG. 1. GROWTH OF DENT CORN IN GREENHOUSE, FIFTY-SIX DAYS OLD

Table 6 and figure 1, illustrating the yield of the dry weight of the corn plants from each pot, show that the addition of an easily soluble nitrate operated to increase plant growth. However, the increase due to the addition of the nitrate was not nearly as large as the increase due to the addition of dried blood to the soil.

The crude petroleum did not seem to decrease the growth of the corn until about 80 cc. to 90 cc. had been applied to each pot.

PURE CULTURE STUDIES

Although several organisms persistently appeared in the petri plates from soil treated with crude petroleum, one type was a great deal more prominent than any of the others. A large number of cultures of this organism were isolated from different soils and studied culturally, morphologically and physiologically.

In this work the outline recommended by the 1920 Report of the Committee on Standard Methods of the Society of American Bacteriologists was followed.

The index number of the organism according to this chart is 5331-52220-1233.

In most respects the culture corresponds very closely to the *Mycobacterium hyalinum* described by Sohngen (5) as a bacterium capable of breaking down and utilizing such compounds as benzene, paraffin and petroleum.

Morphology

Form. The organism is a clear cut rod about three times as long as wide. Long chains of 6 or 8 organisms are occasionally found. Although the growth on agar is slimy and gives indication of capsule formation, none has ever been observed.

Size. The organisms are very uniform in size, being about 1 by 3 microns.

Motility. No motility has been observed.

Spore formation. Spores are not formed.

Staining properties. The organism stains readily but not at all uniformly. Heavily stained granules are usually observed at either end of the rod with Loeffler's Methylene Blue. It is Gram negative.

Cultural characteristics

Agar colonies. Colonies on agar develop rather slowly, are medium sized, about 3 to 5 mm. in diameter, elevated, amorphous and glistening.

Agar slant. Growth on agar slant resembles that of the agar colonies, being elevated, glistening and white. It spreads over the surface of the agar and in old cultures tends to form heavy folds or wrinkles of a light yellow hue.

Agar stab. Growth on the surface is in the form of a shiny glistening drop with very slight growth down the line of inoculation.

Gelatin colonies. Colonies resemble the agar colonies very closely. There is no liquefaction of the gelatin.

Gelatin stab. No liquefaction occurs and the growth is rather slow, being slimy and glistening on the surface and very scant along the line of inoculation.

Bouillon. Cloudy growth occurs throughout with the formation of a slight ring around the surface.

Potato streak. Growth is elevated and glistening white, later turning light cream color.

Physiological characteristics

Arabinose, sucrose, lactose, maltose, raffinose, inulin, and glycerin are not fermented. Dextrose is fermented with the production of slight acidity and no gas. Levulose and mannite give rather high acidity, but no gas formation.

Starch is hydrolysed slowly.

Ammonia is formed from urea, but is not formed from casein, dried blood, gelatin, peptone, egg albumin or cotton seed meal.

Nitrates are reduced to nitrites without the formation of gas.

The organism grows best in the presence of oxygen, but is able to grow in the absence of oxygen to a slight extent.

SUMMARY

1. The soil flora is changed remarkably by applications of crude petroleum. Most types of bacteria are inhibited by the action of the crude petroleum, but some few types are very greatly stimulated by its action. Mold growth is not inhibited by the action of the crude petroleum.

2. Ammonia production in the soil is lowered slightly by applications of crude petroleum. The ammonia produced in the soil is probably the result of mold growth and not bacterial action as the bacterial types favored by the crude petroleum are not able to form ammonia from organic material.

3. When first applied, nitrate production in the soil is completely inhibited by the crude petroleum. The inhibitory action lasts over a varying period of time, depending upon the size of the application, and is followed by a period of rather slow nitrification, which gradually becomes more intense.

4. The data in regard to crop growth are not conclusive, but the indications are that small applications of crude petroleum to the soil do not injure its crop-producing power. Larger applications have a detrimental influence partly because of their effect on the physical condition of the soil.

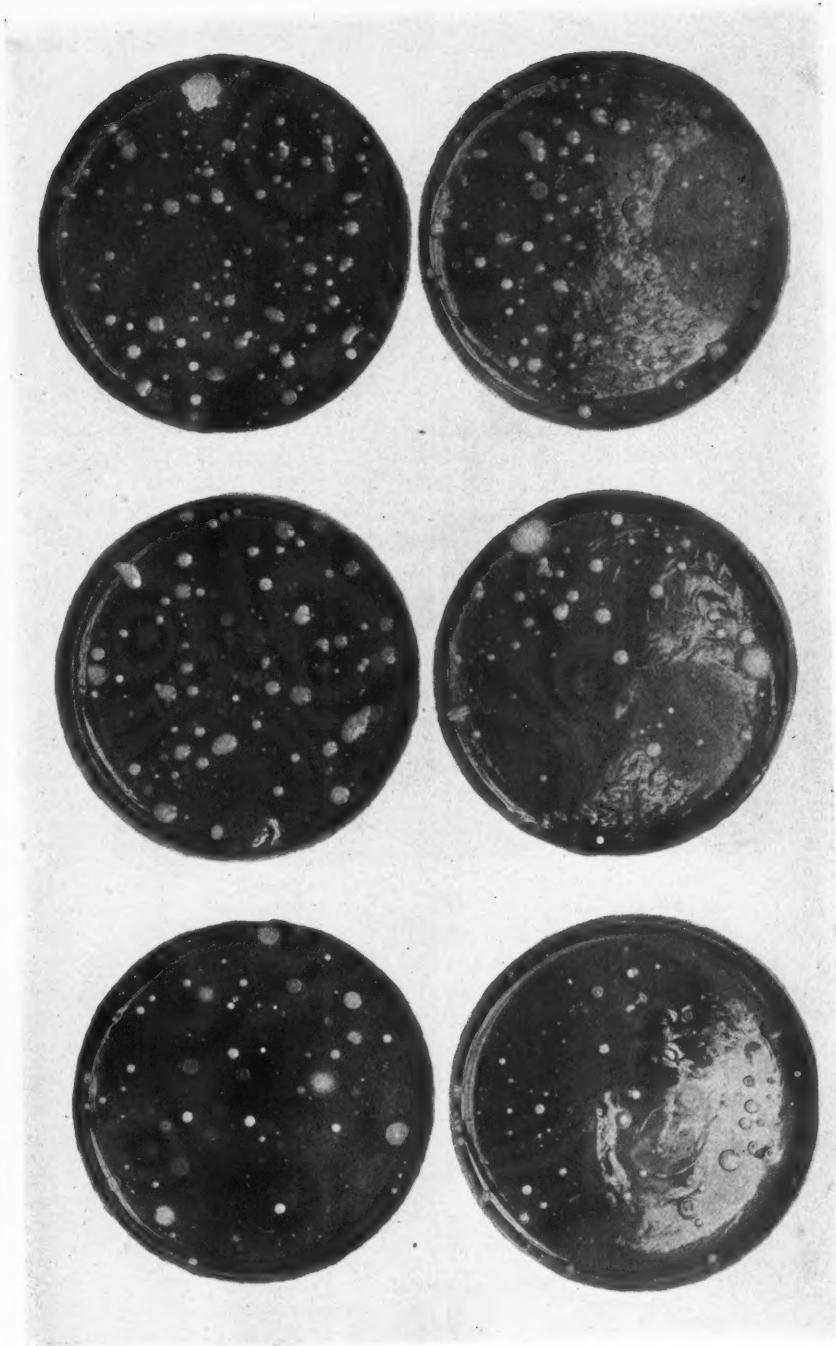
5. It seems that crude petroleum when incorporated in soil is gradually broken down into simpler products and the effect of its presence is no longer apparent.

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PLATE 1
PETRI PLATES INOCULATED WITH 1 CC. OF A 1-100,000 DILUTION OF SOIL

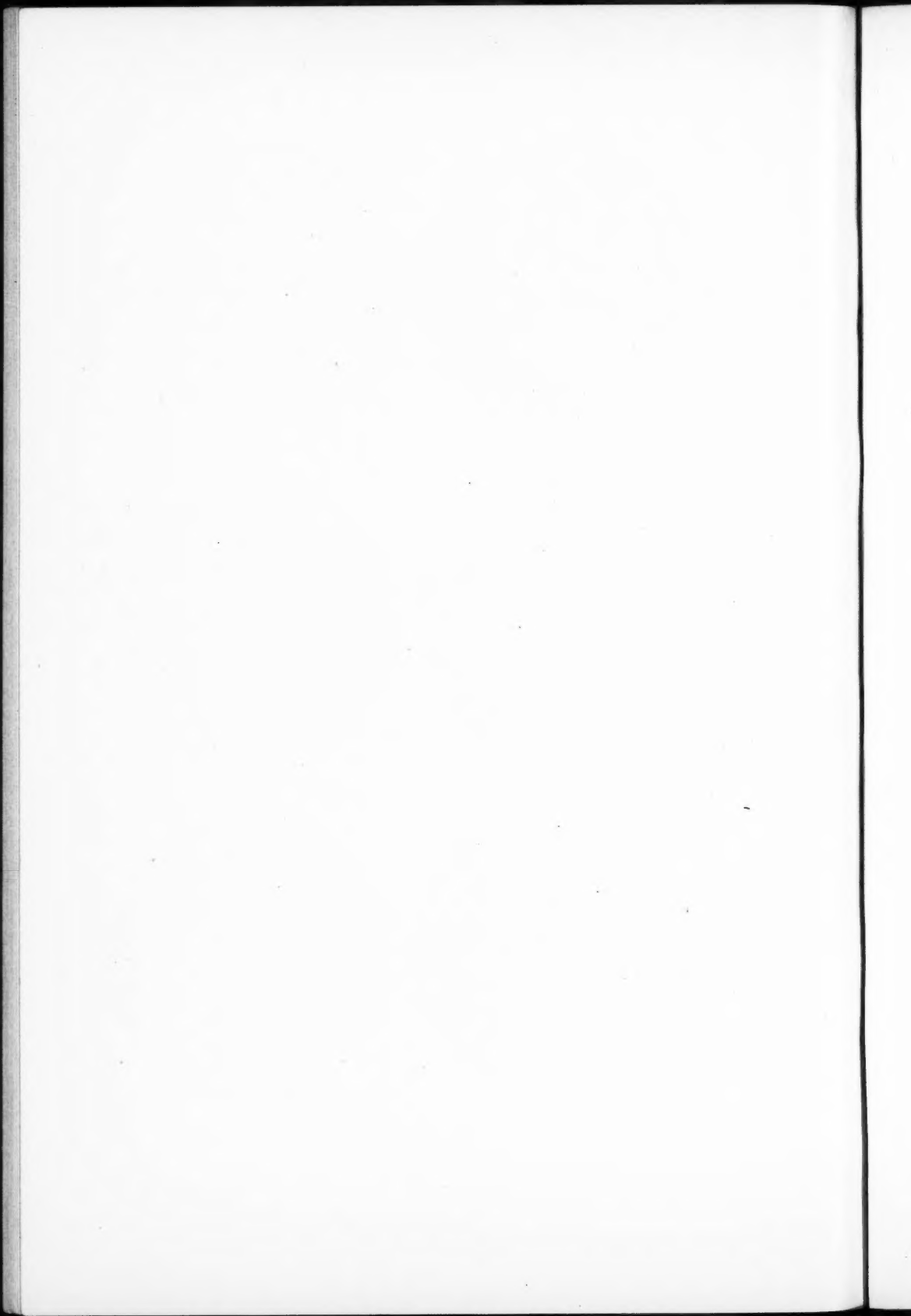
EFFECT OF PETROLEUM ON SOIL FLORA
I. L. BALDWIN



5 cc. oil per 100 gm. soil

2 cc. oil per 100 gm. soil

No oil



ACID PHOSPHATE PRODUCTION BY THE LIPMAN PROCESS:
I. EFFECT OF MOISTURE CONTENT OF SULFUR-FLOATS-
SOIL MIXTURES ON SULFUR OXIDATION ACTIVITIES¹

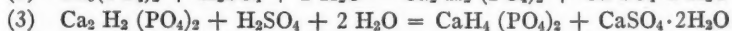
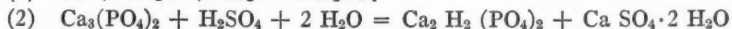
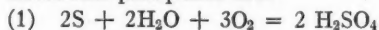
JACOB S. JOFFE

New Jersey Agricultural Experiment Stations

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The Lipman process of mixing rock phosphate, sulfur and some other inert material for the purpose of making acid phosphate is based upon the phenomenon of the oxidation of sulfur by microorganisms, chiefly *Thiobacillus thiooxidans* (6, 10). A number of investigators (1, 2, 3, 4, 13), have followed up the pioneer work of Lipman and his associates (8, 11). Their achievements have been negligible in comparison with the hopes and faith voiced by Lipman in his early work and boldly maintained by McLean (11). All, however, felt the possibilities of making acid phosphate by a bacterial process, eliminating thereby first the sulfuric acid production for acid phosphate production, and secondly the elimination of the acidulation process of the rock phosphate. The idea may sound far fetched but it is the firm belief of the writer that this is possible and the experiments reported below will show how the process may be developed. The chief failure of the investigations including even those of Rudolfs (12) conducted in France almost parallel with the writer's work, is their failure to appreciate some of the fundamental reactions involved in the oxidation of sulfur by microorganisms. It is true that these reactions were overlooked even by Lipman and McLean, but their work was pioneer work. Yet a thorough examination of their work will show how comprehensive in scope it was, how systematically it was attacked, how thoroughly some of the features were looked into, and how many phases of the work were touched. Their followers simply checked up their work and as a result very little progress has been made toward attaining a way of making acid phosphate by the Lipman process.

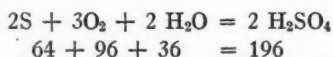
In the case of the sulfur-floats mixtures, the reactions that take place when sulfur is oxidized to sulfuric acid and when this acid reacts with the tricalcium phosphate are:



¹Paper No. 103 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

This paper will appear in Rutgers College Studies, vol. 1.

Equation (1), when analyzed from a stoichiometric standpoint, tells us that with the progress of the sulfur oxidation water is taken out from the system for the formation of the sulfuric acid. None of the workers on the problem of securing an ideal mixture for the rapid transformation of the insoluble phosphates have taken this factor in consideration. Their procedure was to weigh the cultures weekly or at other intervals of time, consider the loss in weight as the measure of water lost by evaporation, and add only this much water for maintaining a proper moisture-content. In reality, however, there was an additional loss of culture-moisture equivalent to the weight of the water used for building up the sulfuric acid molecule and the water of crystallization in gypsum plus the weight of the oxygen necessarily introduced for the formation of the sulfuric acid.



Thus from 64 units of sulfur 196 units of sulfuric acid are formed, or for every gram of sulfur, 3.02 gm. of sulfuric acid, a gain in weight of more than 2 gm. for every gram of sulfur oxidized. Also, as indicated, in the second and third equations, one of the end products of the reaction is gypsum, each molecule of which holds two molecules of water of crystallization. This again causes the system to gain weight and minimize the apparent loss by evaporation. The cultures, therefore, suffered a constantly progressing deficiency in moisture and oxidation was necessarily retarded. Experiments conducted by the writer with a view of studying the gain in weight in cultures of progressive sulfur oxidation proved this theoretical consideration beyond doubt. In sand cultures a gain very close to the theoretical was obtained while with some mixtures of rock phosphate, sulfur and soil the gain at times was below the theoretical probably due to the liberation of CO_2 from some of the carbonates present in the mixtures. Thus the moisture-content of the mixtures is a function of the sulfur oxidized.

Another point which the first equation brings up is the oxygen supply in the process of sulfur oxidation. This point has been appreciated by Lipman and McLean; but Rudolfs (12) contrary to all expectations, from a theoretical point of view, found that aeration had no stimulating effect on the oxidation of sulfur. It might be suspected that some other factor, perhaps the one of the proper moisture-content, was the cause of the failure. Experiments on the effect of aeration have already been reported elsewhere (6).

The reactions involved in the conversion of rock phosphate into soluble forms by means of acid as represented empirically in equations (2) and (3), belong to the heterogeneous system and have been studied very little. Throughout the work it was noticed that the cultures reached a hydrogen-

ion concentration as expressed by the Sørensen figure pH 2.8. A test of the chemically pure primary calcium phosphate as well as that of the commercial acid phosphate also gave a pH 2.8. Naturally the speeding up of the initial stage of incubation in order to reach such a pH was considered of importance. Experiments were conducted to see whether additions of sulfuric acid at the start will bring about such a condition. These experiments have been reported already (7).

An important step towards the successful accomplishment of making acid phosphate by the Lipman process was the building up of mixtures of rock phosphate, sulfur and some other inert material, whereby the total and the available phosphate content should both be high.

EXPERIMENT 1. EFFECT OF MOISTURE CONTENT

A mixture in the proportions of 15 gm. of rock phosphate flour, 5 gm. of sulfur and 80 gm. of greenhouse soil was distributed into small earthenware pots in 400-gm. portions. Pots 1 and 2 received water equal to 50 per cent of the total moisture-holding capacity; 3 and 4, 60 per cent; 5 and 6, 75 per cent; 7 and 8, 50 per cent. With the exception of pots 7 and 8 the moisture-content was kept up, by weekly determinations to the amount added at the start. Pots 7 and 8 were weighed carefully, and the loss in weight was considered as the loss by evaporation. Results are given in table 1.

The results presented are very striking and bring out several interesting points. First, McLean's (11) results that 50 to 60 per cent saturation of the mixture is the best are confirmed. Pots 1 and 2 show this very clearly. Second, the failure of some investigators to get results even with 50 per cent saturation is due to the erroneous calculation of the moisture lost by evaporation, as pointed out above. After the third week the importance of the moisture factor begins to show, although the oxidation of sulfur is still going on, as indicated by the gradual accumulation of soluble phosphorus. The limiting factor at such a moisture-content is not of a direct nature. In another part of this work to be reported later the physico-chemical principles of the course of conversion of insoluble phosphates into soluble forms are expounded and it is pointed out that in such a system the speed of diffusion of the reacting substances and the amount of contact of the reacting substances play an important rôle. In cultures 7 and 8 there may have been sufficient moisture for the metabolic processes of the organisms responsible for the oxidation of the sulfur, but the diffusing power and amount of contact is lowered. Third, cultures 3 and 4 lagged behind until the pH went down to 2.8, but gained rapidly after reaching this point. It has been pointed out elsewhere (6) that up to the pH 2.8, the sulfur-oxidizing organisms have to compete with other groups of microorganisms. It is very likely true that at a 50 per cent saturation the sulfur-oxidizing flora is at an ad-

TABLE 1
Effect of moisture content on availability of rock phosphate in composting it with sulfur and soil

POT NUMBER	AFTER 1 WEEK		AFTER 2 WEEKS		AFTER 3 WEEKS		AFTER 4 WEEKS		AFTER 5 WEEKS		AFTER 6 WEEKS		AFTER 7 WEEKS		AFTER 8 WEEKS	
	Reac- tion	Soluble P in 100 gm. of mixture	Reac- tion	Soluble P in 100 gm. of mixture	Reac- tion	Soluble P in 100 gm. of mixture	Reac- tion	Soluble P in 100 gm. of mixture	Reac- tion	Soluble P in 100 gm. of mixture	Reac- tion	Soluble P in 100 gm. of mixture	Reac- tion	Soluble P in 100 gm. of mixture	Reac- tion	Soluble P in 100 gm. of mixture
	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.	pH	mgm.
1	5.0	32.1*	3.4	43.1	3.2	69.2	2.8	92.8	2.8	104.9	2.6	128.7	2.6	149.7	2.4	171.3
2	5.0	31.3	3.4	42.7	3.0	69.9	2.8	94.7	2.6	111.7	2.6	134.6	2.6	150.4	2.4	170.1
3	5.2	30.4	3.8	34.0	3.6	58.7	3.0	89.2	2.8	112.3	2.6	142.7	2.4	169.3	2.2	189.4
4	5.2	29.6	3.8	36.2	3.4	57.9	3.0	90.3	2.8	116.4	2.6	139.6	2.4	168.1	2.2	186.2
5	5.6	28.7	4.2	30.6	4.0	42.1	3.8	43.7	3.8	41.9	3.4	54.2	3.0	59.7	2.8	69.3
6	5.6	27.9	4.4	31.0	4.0	41.6	3.8	44.3	3.8	42.7	3.0	59.6	3.0	61.3	2.8	70.7
7	5.0	32.1	3.4	43.0	3.0	63.6	2.8	84.2	2.8	91.6	2.8	96.7	2.8	103.4	2.6	119.1
8	5.0	32.2	3.2	44.0	3.0	61.7	2.8	83.9	2.8	87.2	2.8	94.9	2.8	101.3	2.8	111.7

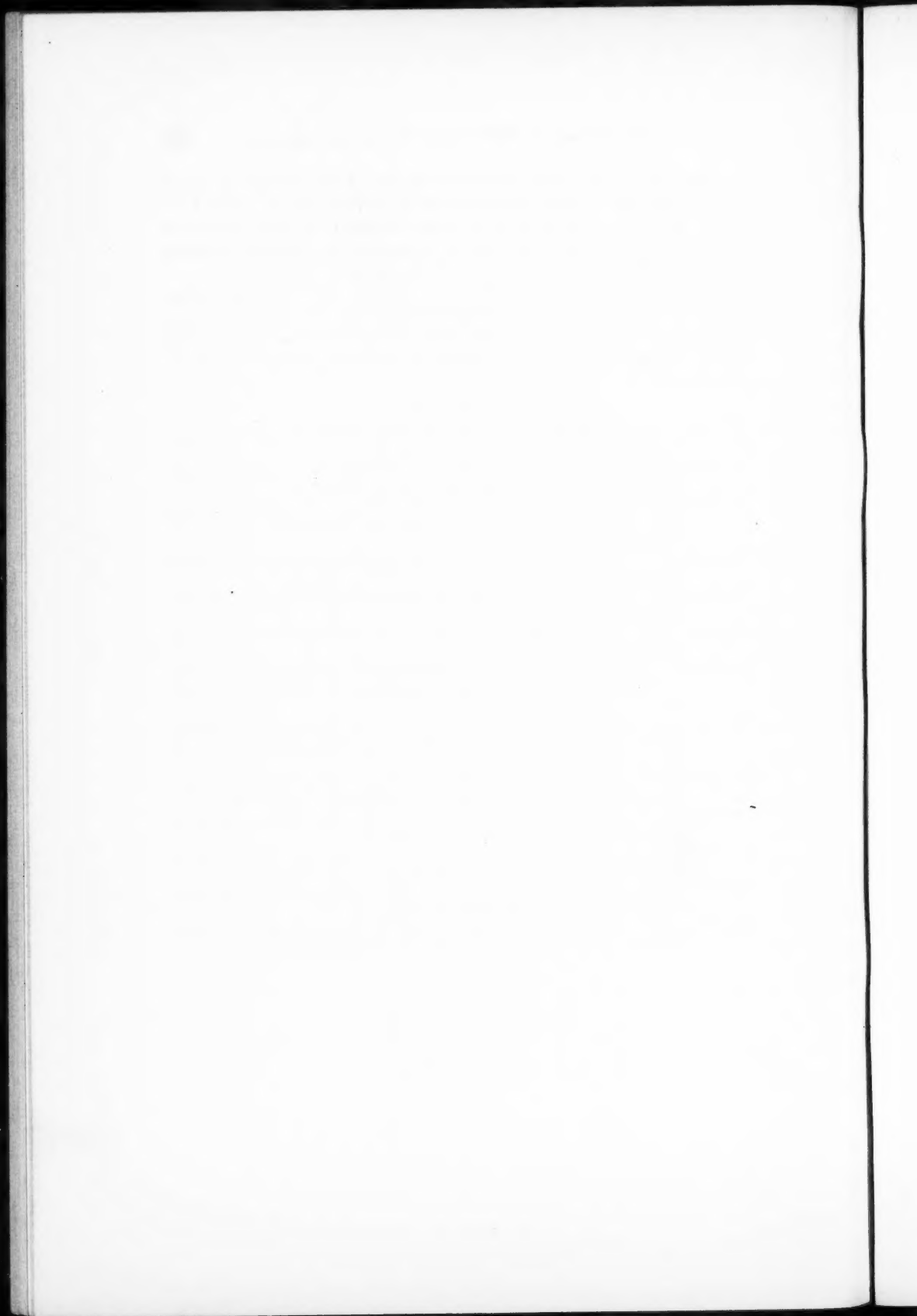
* Total phosphorus content of mixture at start was 203 mgm. per 100 gm. of mixture.

vantage over others, while after reaching the pH 2.8 the field of action is clear and then a higher moisture-content is just what is required for proper diffusion. Fourth, a more nearly saturated condition, as the 75 per cent saturation was in this case, is detrimental to the sulfur oxidizing flora, especially in the early period of incubation.

From this experiment, therefore, it appears that for ideal sulfur oxidizing conditions the cultures should be started with a moisture-content of 50 per cent saturation, and after the reaction reaches a reaction of pH 2.8 the moisture-content should be gradually raised to a 60 per cent saturation.

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A NEW METHOD OF MECHANICAL ANALYSIS OF SOILS

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From the beginning of scientific work on soils it has been recognized that a mechanical analysis is a necessary part of any complete soil description. There is a tendency for investigators in soils to seek for generalized characteristics, such, for example, as the effective radius, the moisture equivalent, and the hygroscopic coefficient. It is evident, however, that the ultimate solution of many of the problems in soil physics and chemistry must depend upon information which will come with a greater knowledge of the mechanical states and processes of the soil in their microscopic detail. Although many serious efforts have been made to perfect a suitable method of mechanical analysis, it can scarcely be said that satisfactory results have been obtained.

The elutriation and sedimentation methods which have been described are so laborious and time-consuming that their use for obtaining the complete frequency distribution curve is quite out of the question except in certain special cases. The standard methods of this type have been studied and compared by Joseph and Martin (4) who recommend the use of sodium carbonate as the deflocculating agent and gravity sedimentation as the most convenient means of effecting the separation. The various methods of obtaining the distribution curve have been summarized by Wightman and Sheppard (12) who also submit some interesting results with silver halide suspensions obtained by two optical methods. Since developing the method described in this paper, we have had access to the recent papers of Odén (6, 7, 8). He has elaborated the fundamental idea of the simple sedimentation method of Schloessing (11) and devised an ingenious procedure for obtaining the complete distribution curve of soils. He suspends a balance pan near the bottom of a cylinder containing the soil suspension and measures the rate of settling of the material on the pan. From the "accumulation" curve so obtained the distribution curve is calculated. The method we propose has been developed primarily as a result of the need, long felt at this Station, for a simple means of determining the amount of very fine material in soils, particularly the particles smaller than one micron in diameter. This method is also applicable to the fine sand and silt. The authors feel that it offers many advantages over other methods.

APPARATUS AND TECHNIQUE

The method is simple. It consists in shaking a dilute, fully deflocculated soil suspension in a cylindrical vessel, placing the latter in an upright position, and determining the concentration as it changes with the time at measured distances below the surface. We have found an ordinary Oldberg percolator, with a centimeter scale on the outside, convenient for this purpose. The sample to be analyzed is drawn off from below through a permanently fixed multiple-intake pipette with the openings turned in a horizontal direction, as illustrated in figure 1. The capacity of this pipette is made small in comparison with the size of sample to be drawn off and the rate of flow is controlled by a glass nozzle at the outlet. We make a practice of discarding the first small fraction standing in the pipette together with a small additional amount used for washing. The percolator we have used is 40 cm. high by 8.5 cm. in diameter, and the pipette with nine intakes has a capacity of about 2 cc. As indicated below, the limit of precision is in the sampling and weighing, and it is therefore necessary to use every precaution for accuracy in these operations. It is possible that the nephelometer may be used with greater speed and precision for determining the suspension density when only very small particles are present, and we hope to investigate its use for this purpose.

We have found that when the rate of flow of the sample is about 15 cc. per minute, the currents set up in the cylinder are not strong enough to cause appreciable mixing, and it is possible to go on sampling at frequent intervals without shaking the suspension after each sampling. This procedure has been successfully adopted when all the particles with a radius larger than 5μ have had time to fall below the intake of the pipette. The larger particles, however, fall so rapidly that the sample must be taken more quickly when they are present in order that the ratio of the time of sampling to the total time of settling may be small and in order that the stream lines may be strong enough to carry the large particles into the sampler. We therefore use as high a column of liquid as possible at first, omit the nozzle, and shake the suspension after each sampling. The position of the surface is read before and after each portion is drawn off.

The problem of attaining and maintaining a completely deflocculated condition in the suspension is vital to this method. Odén (7, p. 332) recommends a concentration of 0.007 to 0.02 per cent of ammonia in the liquid after the usual preliminary shaking. Arid soils generally contain so much calcium carbonate and soluble salts that his method fails in its purpose. We have therefore adopted with promising success, the recommendation of Joseph and Martin (4), of using sodium carbonate as the deflocculating agent. This salt diminishes the concentration of the calcium ions and at the same time supplies hydroxyl ions to charge the soil particles. It would seem from our limited experience that the best concentration to use is about 0.015 to 0.03 per cent. With smaller and larger amounts flocculation has occurred. We are investigating this point, and another paper is in process on this question.

It is also important in this method to adjust the suspension density within certain limits. On the one hand the concentration must not be so high that the particles interfere with each other appreciably in their fall, and on the other hand it must not be so low that a suitable sample does not contain enough solid material to weigh accurately. Odén (8) states that 1 per cent is not too high a concentration. Our experimental data indicate that about 0.2 per cent of material below 5μ radius is appropriate, but this point needs further investigation.

Since the rate of fall of a particle is inversely proportional to the viscosity of the liquid, and since, as Robinson (10) points out, the latter is increased about 30 per cent by a decrease in temperature of 10° , it is obvious that a large error may be introduced through a failure to control the temperature. This error may be further increased by mixing due to convection currents caused by unequal heating of the system. Instead of using a thermostat we have set up our apparatus in a deep basement room in which the temperature has been found to vary but slightly. The tubes have also been properly insulated in order to prevent the influence of local temperature changes.

DISCUSSION OF THEORY

Physical basis

This method involves the assumption that the particles fall as individuals at a constant rate irrespective of the presence of other particles. It classifies the soil into fractions according to their rates of fall through the liquid and, in order to approximate the size of these particles we make use of Stokes' equation

$$v = Cr^2 \quad (1)$$

where

$$C = \frac{(2/9) g (\rho - \rho')}{\eta}$$

In this equation v is the rate of fall of spherical particles of radius r and density ρ in a liquid of viscosity η and density ρ' . Knott (5) has pointed out that the use of Stokes' equation in this sense serves only to define the effective radius.

If any irregular particle is thought of as being molded into a sphere, the radius of the sphere may be regarded as the radius of the particle. This will differ slightly of course from the value determined by the above equation, not only because the particle is not spherical in shape but also because its density may differ from the mean value for the entire sample. These difficulties are inherent in any sedimentation method. We shall designate the value of r that satisfies equation (1) as the "equivalent" radius. Odén (6) assumes that the density of the sample is constant in his definition of "effective" radius. It should be noted that the term "effective" radius is used in soils literature in another sense, and the term "equivalent" radius is probably preferable in this connection.

It is well known that the density of the mineral material of a soil is practically independent of the size of the particles so that the variation in density throughout the sample will be small. The organic matter offers another problem that deserves special study. Odén (6), Hall (3), and other investigators (2, 12) have concluded that the rate of fall of an irregularly shaped particle is not materially different from that of a sphere of the same volume and density.

The upper limit of application of Stokes' law is given by Allen (1):

$$r^2 = \frac{9 \eta^2}{2 g \rho' (\rho - \rho')}$$

and represent a radius of about 85μ in the case of ordinary soil particles. The lower limit has not been determined, but Perrin (9) has shown that the law holds for particles to about 0.2μ radius.

The calculation

The suspension density at any point ah (fig. 1) beneath the surface is due to the presence of a large number of different sized particles, and the decrease in this aggregate concentration is due to the disappearance one by one of these separate species. The concentration of the particles of any given size at this point will remain constant until those particles (of this size) which were originally at the surface have had time to fall through the distance ah . The concentration of this species will then decrease abruptly to zero. In other words each species of particles may be thought of as falling as a solid column, independently of all the others.

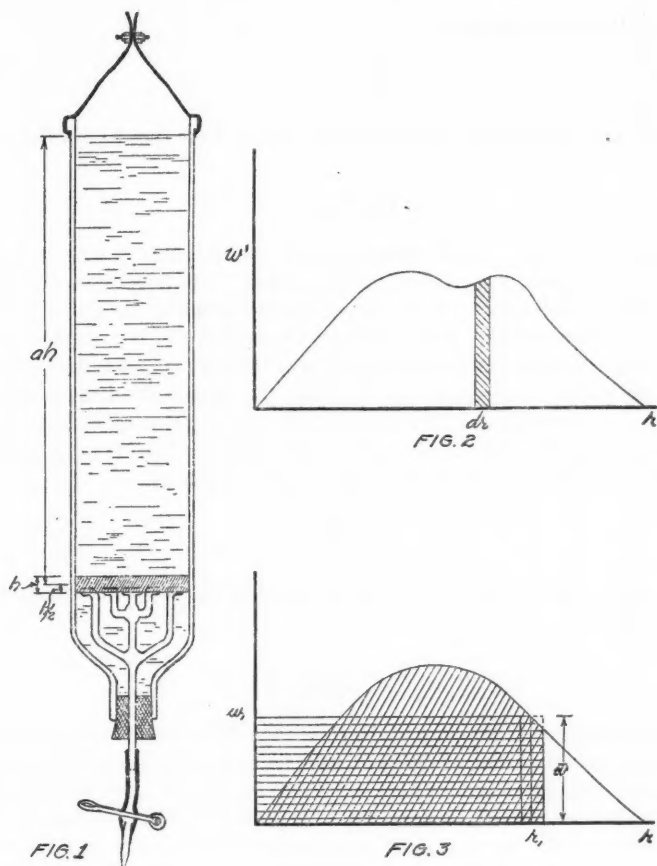
The difference between two successive measurements of the suspension density q' will represent the amount of a fraction whose limiting dimensions are given by the radii corresponding to the two times at which the measurements are taken. If therefore the relation between q' and t is obtained experimentally, we may calculate the equivalent radius by Stokes' law:

$$r = \sqrt{\frac{ah}{Ct}} \quad (1')$$

and read at once from the graph representing the $(q'-r)$ relationship the amount of the sample lying between any two values of r . If we wish to express the suspension density as a fraction part of the original concentration q_0 , we may plot the ratio $\left(\frac{q'}{q_0}\right) = q$, against the radius r , and read the percentage of any fraction from the graph.

If the weight (w') of particles of radius r in the sample is represented graphically as a continuous function of r (see figure 2) we may write:

$$dq' = \left(\frac{w'}{L}\right) dr \quad (2)$$



where L is the volume of the liquid in which the soil is suspended. Or, if W is the total weight of the sample, we may write for convenience:

$$\left(\frac{w}{W}\right) = w, \left(\frac{q'}{q_0}\right) = q, \text{ and } \left(\frac{W}{L}\right) = q_0$$

equation (2) will then become

$$\frac{dq}{dr} = w \quad (3)$$

Using this equation we may calculate the $w-r$ curve from the slope of the $q-r$ curve.

Precision

In figure 4 is shown a group of hypothetical curves (series 2) for which the equations are known, representing in their general form possible $w-r$ distribution curves, and in series 1 the $q-r$ curves from which they were obtained by the above transformation. From the form of equation (3), it is obvious that the measure of precision in the determination of the $w-r$ curves is the same as that of the slopes of the original series 1 and the magnitude of the probable error may be estimated from the following. If n cc. are drawn off in the sample to be used for the determination of q' , the total weight m of soil remaining after the water has been evaporated will be given by the equation:

$$m = \frac{nq'}{L}$$

If e represents the probable error of the weighing of the sample, the fractional error E will be:

$$E = \frac{e}{nq'/L}$$

In the determination of the slope of the curves the difference in ordinates must be measured corresponding to different values of r , but inasmuch as the error in the latter may be controlled almost entirely by the choice of the scale on which the data are plotted, the error will arise principally from the error in the determination of q' . At the highest point on the curves of series 1 of figure 4, the width of the line is of the order of $\frac{1}{25}$ of the ordinate, so that an experimental error of this magnitude should not fall appreciably off the curve.

In order to sample the suspension it will be necessary to draw off a finite stratum of thickness h (see figure 1), and if h is small the suspension density may be said to vary linearly over this range and the sample to represent the concentration at the middle point of the section. A small error will be introduced by this assumption since the density is not strictly a linear function of the depth over this range. The magnitude of the error may be estimated as follows:

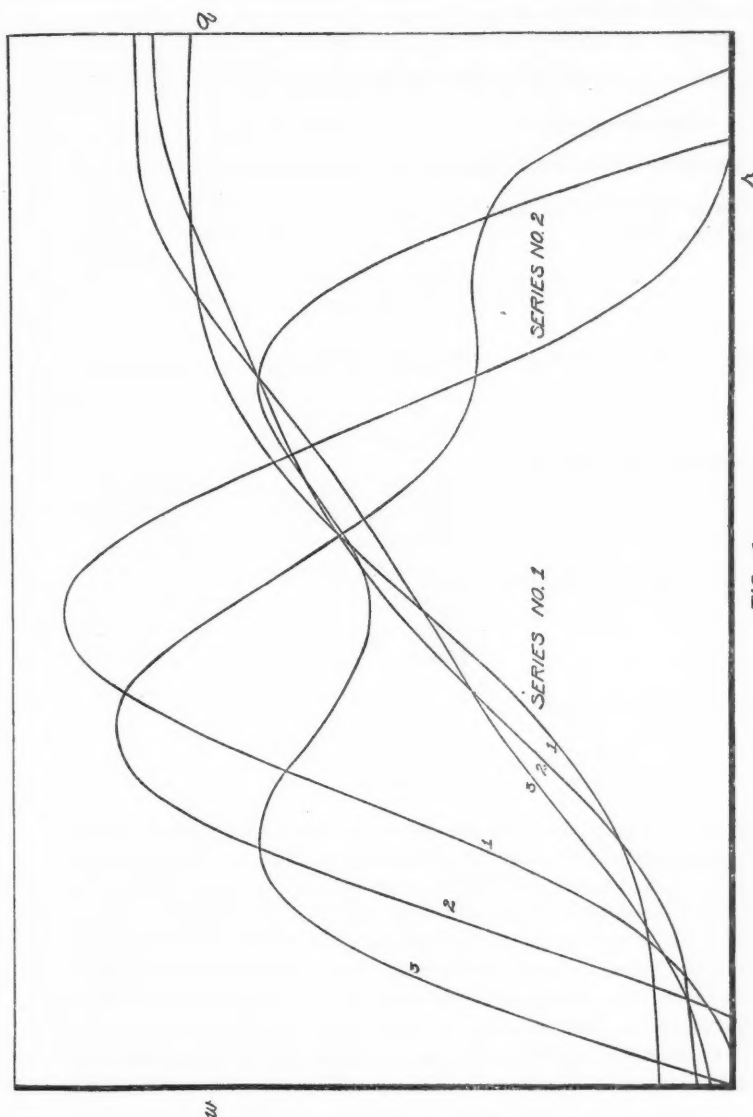


FIG. 4

FIG. 4. ILLUSTRATIVE DISTRIBUTION CURVES (SERIES 2) AND THE HYPOTHETICAL CURVES (SERIES 1) FROM WHICH THEY WERE OBTAINED

These curves serve to indicate the way in which detail in the distribution curves may be suppressed in the experimental curves from which they are calculated.

If r_1 = radius of particle of species vanishing at a distance ah beneath the surface at time t

q_1 = density of suspension at this point at time t

r_2 = radius of particle of species vanishing at point $ah + \frac{bh}{2}$ at time t

where b is a fraction

q_2 = density of suspension at point $ah + \frac{bh}{2}$ at time t

\bar{w} = mean value of w for interval $r_2 - r_1$

We may then write

$$\frac{q_2 - q_1}{q_1} = \frac{\bar{w} (r_2 - r_1) (r_1)}{\int_0^{r_1} w dr (r_1)} \quad (4)$$

By means of equation (1') we may eliminate the ratio

$$\frac{r_2 - r_1}{r_1}$$

by substituting in its place the ratio

$$\frac{\sqrt{a + b/2} - \sqrt{a}}{\sqrt{a}}$$

We may also substitute R for the ratio

$$\frac{\bar{w} r_1}{\int_0^{r_1} w dr}$$

and equation (4) will become

$$\frac{q_2 - q_1}{q_1} = \frac{R (\sqrt{a + b/2} - \sqrt{a})}{\sqrt{a}} \quad (4')$$

From figure 3 it may be seen that the ratio R is equal to the ratio of the rectangular portion to the area of the curve from 0 to the point r_1 . This magnitude will therefore vary through a small finite range not far from unity, whereas the other factor of the right hand member of (4)' may be made as small as desired consistent with other considerations governing the choice of the factor a . The small particles will of course move slowly, and the depth from which the sample is taken must therefore be governed by this consideration. For a value of $a = 10$ cm., this ratio is of the order 0.025 if we give to b the value unity. As a matter of fact b must be less than unity and in the ordinary case will be a small fraction. For $b = 0.1$ this ratio will become of the order 0.0025.

Another source of error which may be significant for large values of r arises from the fact that in securing the sample for analysis the heavier particles may not all follow the stream lines into the pipette, and a certain fractional part of each species may fall below the intake. If we break up the quantity q' into

its component parts, q_1' , q_2' , q_3' , etc., and if we lose an amount α , of species 1, α_2 of species 2, α_3 of species 3, and so on, we shall have for two successive measured values of q' :

$$q'_a = \Sigma \{ (q_1 - \alpha_1) + (q_2' - \alpha_2) + \dots \}$$

$$q'_b = \Sigma \{ (q_2' - \alpha_2) + (q_3' - \alpha_3) + \dots \}$$

In measuring the slope of the curves the difference of these two values of q' will be the value obtained, whereas the correct values will be by $q_1' - q_2' = q_1''$. The ratio of the measured and true values of this difference will therefore be given as

$$\frac{q'_a - q'_b}{q_1' - q_2'} = \frac{q_1'' - \alpha_1}{q_1''} = 1 - \frac{\alpha_1}{q_1''}$$

so that the fractional error is given by the ration, $\frac{\alpha_1}{q_1}$

EXPERIMENTAL RESULTS

To illustrate the method we are reporting the results obtained for two different types of soil, Greenville silty clay loam and Trenton clay. The former is a good agricultural soil containing a large percentage of fine sand and soil and 1.6 per cent organic carbon, while the latter is a very tight clay containing only 0.65 per cent organic carbon. Both soils are similar chemically and contain about 35 per cent of calcium and magnesium carbonates. The water-soluble material in the two soils is given in table 1.

TABLE 1
Analysis of water-soluble material

SOIL	TOTAL SALTS	CO ₂	HCO ₃	Cl	SO ₄	Ca	Mg
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Greenville silty clay loam.....	0.038	None	0.017	0.003	0.004	0.013	0.003
Trenton clay.....	0.126	None	0.057	0.019	0.038	0.008	0.003

The experiment was carried out as described above, the observations being made at time-intervals of from 40 seconds to two weeks, the depth of the sampling varying from 30 cm. to less than 1 cm. The time of each sampling was then corrected to correspond to a standard depth of 10 cm. Ten grams of soil were shaken for 2 hours with 50 cc. of 0.5 per cent sodium carbonate.¹ The Greenville soil was then diluted to 2 liters and the Trenton to 5 liters. Additional sodium carbonate was added to make up the concentrations noted in the legend of the graph of figure 5. Two entirely independent sets of observations are given with each soil. These agree closely in the case of the lighter soil, but the

¹ Subsequent work has shown that two hours' shaking is insufficient to deflocculate certain soils and that oven-dried soils deflocculate more slowly than air-dried soils.

Trenton clay shows some interesting discrepancies. The system with 0.017 per cent sodium carbonate gives low results in the silt, indicating apparently a partial flocculation of this material. The system with 0.041 per cent sodium carbonate behaves normally for a long time, but there eventually appears to be flocculation of the very fine clay, due perhaps to the high concentration of the sodium ion. It should be noted in this connection that another experiment, not recorded here, in which 0.05 per cent sodium carbonate was used showed flocculation of the clay at an earlier stage of the process. This work indicates the need for careful flocculation studies, particularly in dealing with soils in which soluble salts are present.

As a control on this method, we have made a number of sedimentation separations of these soils by a modification of the method of Joseph and Martin (4), using an 8-ounce bottle to hold the sample and a siphon with the intake turned upward to make the pouring. Individual 2-gram samples were used for each separation, and the sediment remaining when the decantation was complete was dried and weighed. Duplicate determinations almost invariably agreed so perfectly that we consider the method thoroughly reliable. The results thus obtained have been superimposed on the curves obtained by the new method, and it will be observed that the concordance is very good over the range compared, but that the new method gives information concerning a wide range of particles which are too small to be studied by the sedimentation method.

GRAPHICAL REPRESENTATION

The two series of curves of figure 4 illustrate graphically the relation between a function and its derivative and apply to any choice of independent variable.

In plotting our experimental data we have used the ratio $\frac{q'}{q_0} = (q)$ as ordinate and the logarithm of the time as abscissa. This is done entirely as a matter of convenience so that all the data may be shown on the diagram (8). Since the logarithm of the time is a linear function of the logarithm of the radius, plotting the one is equivalent to plotting the other and avoids any numerical calculation. Only the values of the radius however, are indicated on the graph (see figure 5).

We have also shown the derivative of this $(q-\log r)$ curve on the same diagram. If therefore we generalize the meaning of equation (3), regarding w as the weight of soil corresponding to any function of r , $[f(r)]$, this equation may be rewritten in the form:

$$dq = w_1 df_1 = w_2 df_2 = w_3 df_3 \dots \dots \dots (3')$$

the subscript being used to indicate the choice of independent variable. If

therefore $f_1(r) = r$, $f_2(r) = \log r$, and $f_3(r) = \frac{1}{r}$, we may readily obtain the relation

$$\begin{aligned}w_2 &= w_1 r; w_3 = w_1 r^2 \\w_2 d(\log r) &= w_1 r (dr/r) = w_1 dr \\w_3 d(1/r) &= -w_1 r^2 \frac{dr}{r^2} = -w_1 dr\end{aligned}$$

The physical meaning of the logarithmic graph may be somewhat obscure. Another way of condensing the data for large values of r , is to plot its reciprocal as the independent variable. Although this is less convenient than the other method it has an important physical meaning since the reciprocal of the radius is a measure of the specific surface of the particle.

It should be noted that both the $(w_1 - r)$ and $(w_3 - \frac{1}{r})$ relationships can be obtained from the $(q - \log r)$ curve by a simple calculation. If we let

$$y = q$$

and

$$x = \log_{10} r = \log e r \cdot \log_{10} e$$

$$\frac{dy}{dx} = \frac{dq}{d \log_{10} r} = \frac{r}{\log_{10} e} \cdot \frac{dq}{dr}$$

If

$$R = 1/r$$

$$dR = -\frac{dr}{r^2}$$

and

$$\frac{dy}{dx} = \frac{1}{r \log_{10} e} \frac{dq}{dR}$$

These curves are worked out in figure 6 from the diagrams of figure 5.

ROUTINE MECHANICAL ANALYSES

As a routine method of making mechanical analyses, the procedure here proposed has many advantages over the standard methods. The units of apparatus are cheap and as many as desired can readily be set up. Indeed only standard equipment such as may be found in any soils laboratory need be used. If the soil is put through a half-millimeter sieve after shaking it with the deflocculating agent or if a separate sample is treated in this way it will be possible to obtain all the conventional groups of sizes below this point except perhaps the separation at 0.25 mm. diameter by merely drawing off a few samples of the liquid at certain times. Table 2 gives the rate of settling through a 10 cm. column at 16°C. assuming the density of the soil to be 2.7.

By increasing the height of the column of liquid at first and decreasing it later a convenient and accurate system of timing the samples could readily

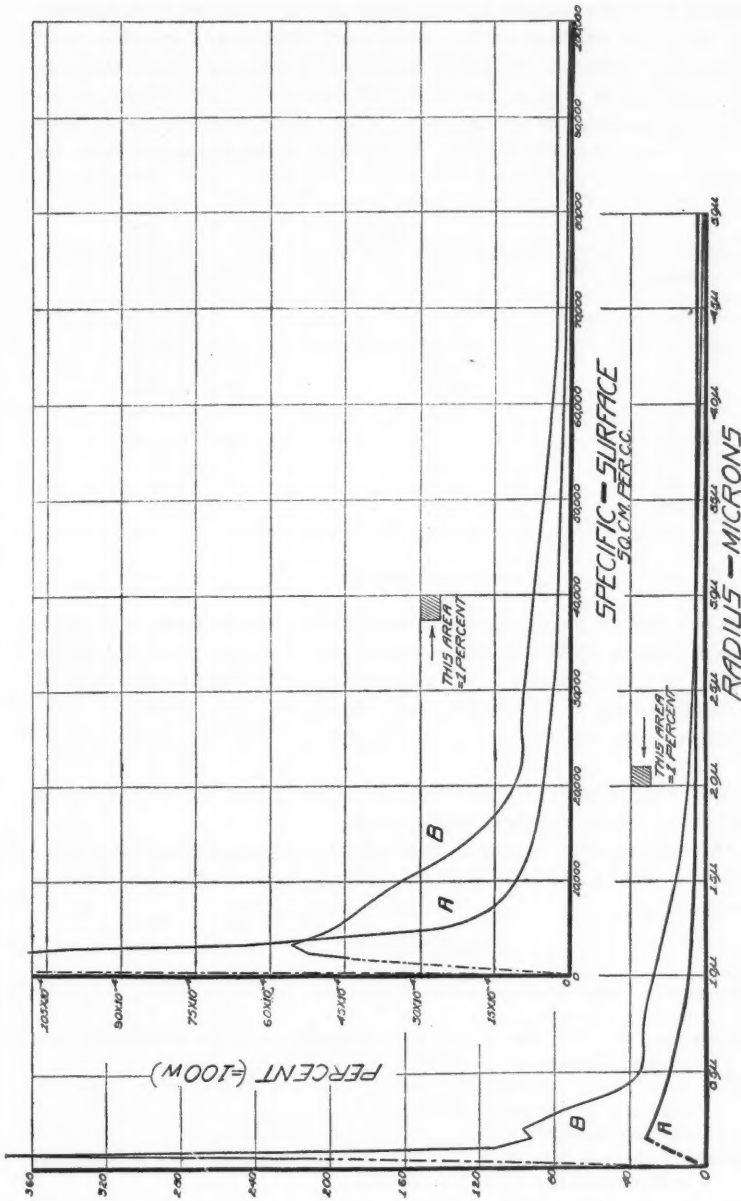


FIG. 6. DISTRIBUTION CURVES FOR TWO SOILS

A, Greenville silty clay loam; B, Trenton clay

be worked out. We estimate that one man, properly equipped with sedimentation units and weighing bottles, could start about eight samples every day, and after obtaining the points indicated in the table finish each set of determinations in three or four days. If fewer points were sought, correspondingly more soils could be analyzed. This output of work would be from five to ten times that obtained by the method of the Bureau of Soils, for example.

TABLE 2
Rate of settling

DIAMETER	RADIUS	TIME TO FALL 10 CM.
mm.	μ	
0.25	125.0	1.9 seconds
0.1	50.0	12.0 seconds
0.05	25.0	48.0 seconds
0.01	5.0	20.0 minutes
0.005	2.5	80.0 minutes
0.002	1.0	8.33 hours
0.001	0.5	33.33 hours
0.0005	0.25	5.55 days

SUMMARY

1. A new method for the rapid and accurate mechanical analysis of soil is described which is applicable to a range of sizes from fine sand to colloidal material. The method may be used either for conventional routine work or for a detailed study of the size-frequency distribution of the particles. Only inexpensive apparatus such as may be found in any soils laboratory need be used.

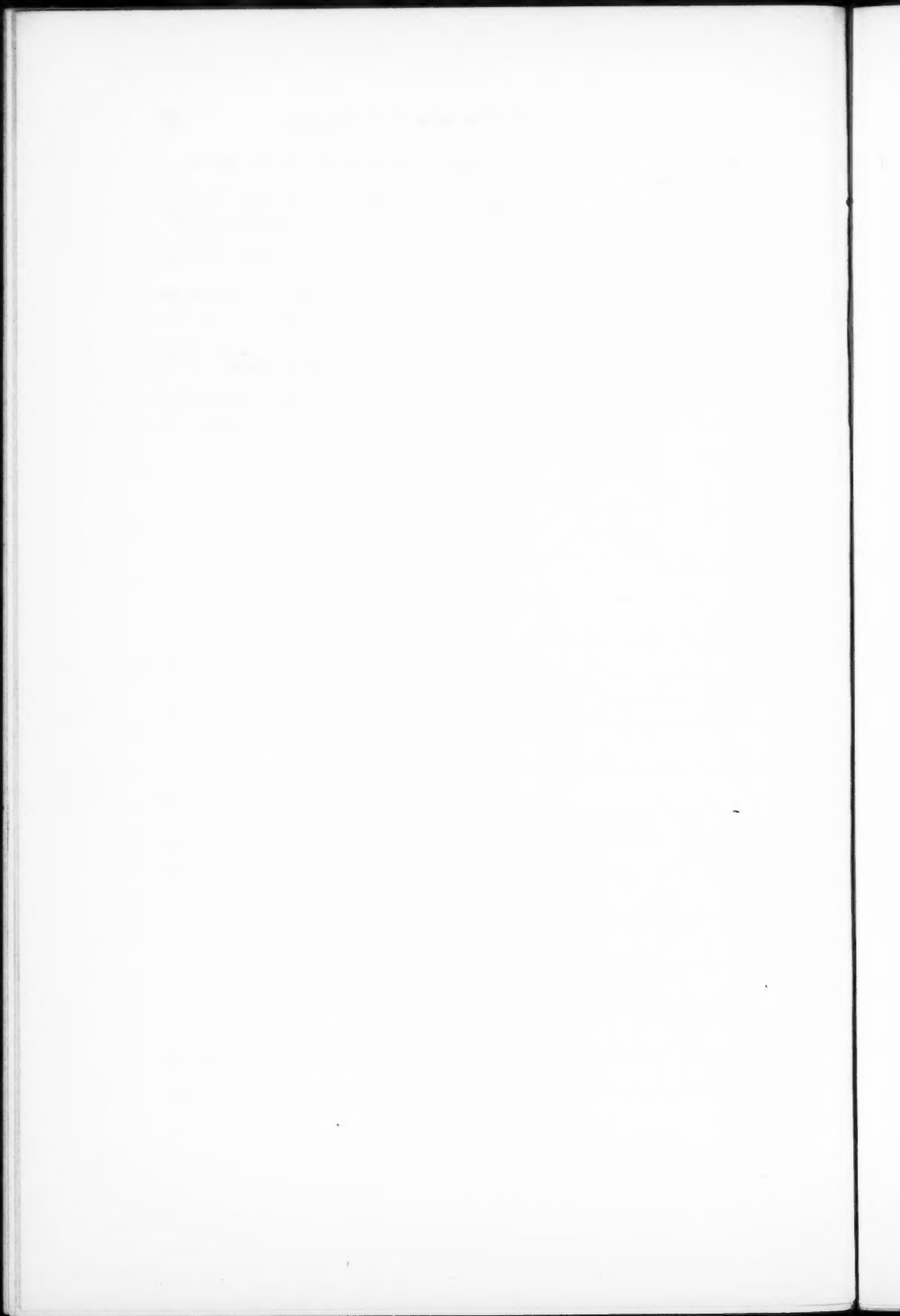
2. Some experimental data are submitted showing that this method agrees closely with a reliable sedimentation method.

3. The method offers a quantitative means of studying flocculation phenomena. It should also yield valuable information concerning the colloidal properties of soil.

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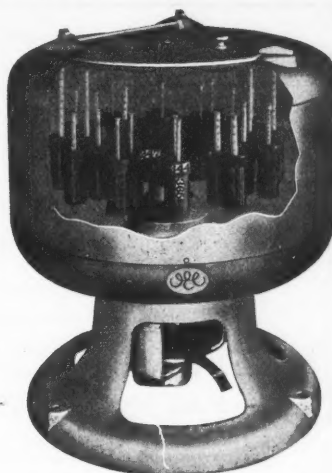
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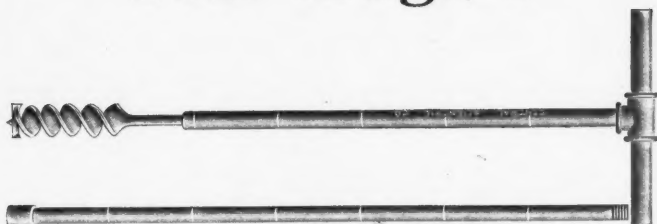
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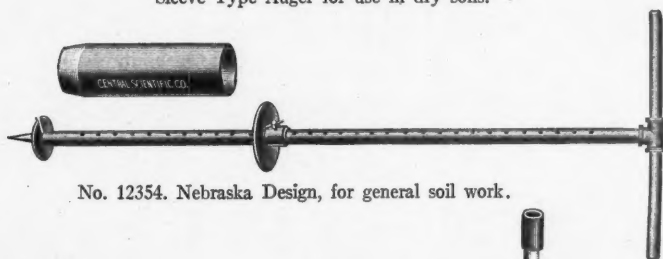
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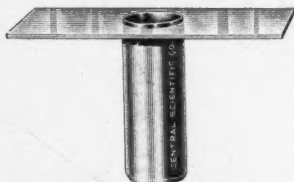
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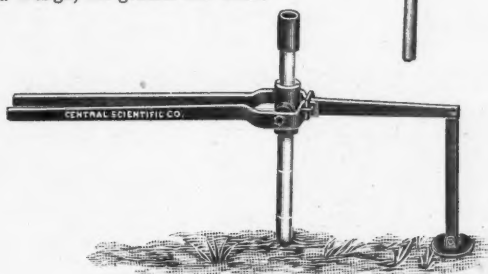
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